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<p>(54) Title: ALPHAVIRUS VECTORS</p> <p>(57) Abstract</p> <p>A modified alphavirus expression vector is provided wherein at least one optimal heterologous splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus, which may be Semliki Forest virus following administration of the vector to a host.</p>		

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TITLE OF INVENTIONALPHAVIRUS VECTORS

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FIELD OF INVENTION

The present invention relates to the field of DNA vaccines and is particularly concerned with modified alpha virus vectors for use in such vaccines.

BACKGROUND OF THE INVENTION

10 Semliki Forest virus (SFV) is a member of the Alphavirus genus in the Togaviridae family. The mature virus particle contains a single copy of a ssRNA genome with a positive polarity that is 5'-capped and 3'-polyadenylated. It functions as an mRNA and naked RNA
15 can start an infection when introduced into cells. Upon infection/transfection, the 5' two-thirds of the genome is translated into a polyprotein that is processed into the four nonstructural proteins (nsP1 to 4) by self cleavage. Once the ns proteins have been synthesized
20 they are responsible for replicating the plus-strand (42S) genome into full-length minus strands (ref. 14). These minus-strands then serve as templates for the synthesis of new plus-strand (42S) genomes and the 26S subgenomic mRNA (ref. 1 - Throughout this application,
25 various references are cited in parentheses to describe more fully the state of the art to which this invention pertains. Full bibliographic information for each citation is found at the end of the specification. The disclosures of these references are hereby incorporated
30 by reference into the present disclosure). This subgenomic mRNA, which is colinear with the last one-third of the genome, encodes the SFV structural

proteins. In 1991 Liljestrom and Garoff (ref. 2) designed a series of expression vectors based on the SFV CDNA replicon. These vectors had the virus structural protein genes deleted to make the way for heterologous
5 inserts, but preserved the nonstructural coding region for production of the nsP1 to 4 replicase complex. Short 5' and 3' sequence elements required for RNA replication were also preserved. A polylinker site was inserted downstream from the 26S promoter followed by
10 translation stop sites in all three frames. An SpeI site was inserted just after the 3' end of the SFV CDNA for linearization of the plasmid for use in vitro transcription reactions.

Injection of SFV RNA encoding a heterologous
15 protein have been shown to result in the expression of the foreign protein and the induction of antibody in a number of studies (refs. 3,4). The use of SFV RNA inoculation to express foreign proteins for the purpose of immunization would have several of the advantages
20 associated with plasmid DNA immunization. For example, SFV RNA encoding a viral antigen may be introduced in the presence of antibody to that virus without a loss in potency due to neutralization by antibodies to the virus. Also, because the protein is expressed in vivo
25 the protein should have the same conformation as the protein expressed by the virus itself. Therefore, concerns about conformational changes which could occur during protein purification leading to a loss in immunogenicity, protective epitopes and possibly
30 immunopotential, could be avoided by plasmid DNA immunization.

In WO95/27044, the disclosure of which is incorporated herein by reference, there is described the use of alphavirus cDNA vectors based on cDNA complementary to the alphavirus RNA sequence. Once
5 transcribed from the cDNA under transcriptional control of a heterologous promoter, the alphavirus RNA is able to self-replicate by means of its own replicase and thereby amplify the copy number of the transcribed recombinant RNA molecules.

10

SUMMARY OF THE INVENTION

The present invention is concerned with modifications to the alphavirus cDNA vectors described in the aforementioned WO 95/27044 to permit enhanced replication of the alphavirus. In the present
15 invention, a heterologous splice site is introduced into the alphavirus replicon sequence, particularly that of Semliki Forest virus (SFV).

Accordingly, in one aspect, the present invention provides an expression vector comprising a DNA molecule
20 complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA, and further comprises a heterologous DNA sequence
25 capable of expression in a suitable host, such as a human or animal host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of
30 a promoter sequence functional in said animal or human host, wherein at least one heterologous splice site is

provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.

The alphavirus molecule is a large molecule and, accordingly, there is a high probability of cryptic splice sites, thereby impairing the replication of the alphavirus and hence its ability to express the heterologous DNA is impaired. By introducing the at least one optimal heterologous splice site in accordance with the present invention into the alphavirus replicon sequence, any splicing is likely to be directed at the heterologous splice site rather than any cryptic splice sites, restores the function of the SFV replicon when removed, and may improve transport of RNA from the nucleus (ref. 6).

In the constructs provided herein, the promoter is placed upstream of the 5'-end of the alphavirus sequence, such that the resultant transcript has an authentic 5'-end, which is required for the efficient replication of the alphavirus RNA replicon.

In addition, there may be provided at the 3'-end of the Semliki Forest virus segment, a hepatitis delta virus ribozyme sequence to ensure proper *in vivo* cleavage at the 3'-end of the sequence. Any other convenient sequence may be employed to achieve this effect.

The heterologous splice site sequence may be provided by the nucleotide sequence of the rabbit β -globin intron II, as described in reference 5. Such heterologous splice site sequence may be inserted into the complement sequence at any convenient location which generates perfect splice junctions. This

precludes replication of the alphavirus, unless it is authentically removed by splicing..

I have identified five suitable sites in the SFV replicon, which are contained within an EcoRV-SpeI
5 fragment of the replicon which is 8010 bp in length (Fig. 3). The first such site is a Ppu-MI site, at position 2719 within the EcoRV-SpeI fragment.

In constructing the modified vectors provided herein, the EcoRV-SpeI fragment is cut with Ppu-MI at
10 position 2719 and made blunt-ended with Mung Bean nuclease, which removes three bases from the SFV sequence. A blunt-ended β -globin II intron, which is 536 bp long, is ligated into the site and replaces the missing three bases with sequence added to the 3'-end
15 of the β -globin intron sequence (Fig. 1).

The other four suitable sites for insertion of the Intron are the PvuII sites at bp 2518, 3113, 6498 and 6872 of the EcoRV-SpeI fragment. Insertion of the Intron is achieved by cutting with PvuII (a blunt end
20 cutter) and the blunt-ended β -globin II intron sequence (Fig. 2) is ligated into one or more of these sites.

In a further aspect of the present invention, there is provided a cloning vector suitable for expression in a host cell of an heterologous DNA
25 sequence, which comprises a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence
30 capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is

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non-essential to replication thereof; a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant transcript had an authentic 5' end; at least one heterologous splice set provided in the complement of the DNA molecule to generate perfect splice junctions in the alphavirus in order to prevent aberrant splicing and an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant mRNA transcript.

BRIEF DESCRIPTION OF DRAWINGS

Figure 1 shows the DNA sequence of the β -globin intron II including three additional nucleotides at the 3'-end thereof (SEQ ID No:1);

Figure 2 shows the DNA sequence of the β -globin intron II (SEQ ID No:2);

Figures 3A to 3C show the DNA sequence of the EcoRV-SpeI fragment of Semliki Forest virus replicon (SEQ ID No:3);

Figures 4A to 4D show the DNA sequence of the pSFV link (SEQ ID no: 4) prepared as illustrated in Figure 5;

Figure 5 shows construction of pSFVlink (11060 bp) from pSFV1 using a linker sequence (SEQ ID nos: 5,6);

Figures 6A to 6D show the nucleotide sequence of plasmid pMP76 (SEQ ID no: 11, prepared as illustrated in Figures 8A to 8D);

Figure 7 illustrates subsections of plasmid pSFV link (see Figure 5);

7

Figure 8A to 8D show the construction of plasmid pMP76 from plasmids pMP53, pMP70, pMP47, pMP55 and pMP71;

Figures 9A to 9B show the construction of plasmids pMP53, pMP54 and pMP55 from plasmid pMP52;

Figure 10 shows the construction of plasmid MP52 from pUC19 using a linker sequence (SEQ ID no: 7,8);

Figures 11A to 11B show the construction of plasmids pMP46, pMP47 and pMP70 from pUC19 and fragment from pSFV link, prepared as seen in Figure 7; and

Figures 12A to 12B show the construction of plasmid pMP71 from plasmid pCMV3.

GENERAL DESCRIPTION OF INVENTION

As discussed above, the present invention provides a modified alphavirus DNA. The alphavirus preferably is Semliki Forest virus. In particular, the present invention provides a cloning vector for heterologous gene expression in a host, such as an animal or human.

The promoter sequence may comprise a promoter of eukaryotic or prokaryotic origin. Suitable promoters are the cytomegalovirus immediate early promoter (pCMV), although other promoters, such as the Rous sarcoma virus long-terminal repeat promoter (pRSV), since, in the case of these and similar promoters, transcription is performed by the DNA-dependent RNA polymerase of the host cell. Additionally, the SP6, T3 or T7 promoters can be used, provided that the cell has first been transformed with genes encoding SP6, T3 or T7 RNA polymerase molecules which are either inserted into the chromosome or remain episomal. Expression of

these (SP6, T3, T7) RNA polymerase-encoding genes is dependent on the host cell DNA-dependent RNA polymerase.

The heterologous DNA insert may comprise the
5 coding sequence for a desired product, which may be a biologically active protein or polypeptide, for example, the heterologous DNA insert may code for HIV sequences, e.g., an immunogenic or antigenic protein or polypeptide, or a therapeutically active protein or
10 polypeptide. The heterologous DNA may also comprise additional sequences, such as a sequence complementary to an RNA sequence which is a self-cleaving ribozyme sequence.

The DNA vectors provided herein may be
15 administered to a host, including a human host, for in vivo expression of the heterologous DNA sequence, in accordance with a further aspect of the invention, in order to generate an immune response in the host, which may be a protective immune response. The DNA vectors
20 may be further formulated into immunogenic compositions for such administration.

BIOLOGICAL DEPOSITS

Certain vectors that contain the Semliki Forest
25 virus replicon and referred to herein have been deposited with the American Type Culture Collection (ATCC) located at 10801 University Boulevard, Manassas, VA 20110-2209, U.S.A., pursuant to the Budapest Treaty and prior to the filing of this application.

30 Samples of the deposited plasmids will become available to the public upon grant of a patent based

upon this United States patent application and all restrictions on access to the deposits will be removed at that time. Non-viable deposits will be replaced. The invention described and claimed herein is not to be
5 limited in scope by plasmids deposited, since the deposited embodiment is intended only as an illustration of the invention.

Deposit Summary

	<u>Plasmid</u>	<u>ATCC Designation</u>	<u>Date Deposited</u>
10	pMP76		

EXAMPLES

The above disclosure generally describes the present invention. A more complete understanding can
15 be obtained by reference to the following specific Examples. These Examples are described solely for purposes of illustration and are not intended to limit the scope of the invention. Changes in form and substitution of equivalents are contemplated as
20 circumstances may suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitations.

Methods of molecular genetics, protein
25 biochemistry and immunology used but not explicitly described in this disclosure and these Examples are amply reported in the scientific literature and are well within the ability of those skilled in the art.

EXAMPLE 1

This Example describes the construction of plasmid pMP76 as outlined in Figures 5, 7, 8A, 8B, 8C, 8D, 9A, 9B, 10, 11A, 11B, 12A and 12B.

5 Plasmid pSFV link was created by restricting plasmid pSFV1 (Gibco) with BamHI. This plasmid was then ligated with a linker (SEQ ID no: 5 and 6) to produce plasmid pSFV link (Figures 4A to 4D, Figure 5).

 Some of the SFV replicon fragments were subcloned
10 by restricting pSFVlink with EcoRV and SpeI and isolating the 890bp EcoRV-SpeI fragment. This fragment was then restricted with EcoRI and the 1906bp EcoRV-EcoRI, the 1578bp and 3627bp EcoRI-EcoRI and the 899bp EcoRI-SpeI fragments isolated (Fig.7).

15 The 1909bp EcoRV-EcoRI SFV fragment was cloned into EcoRV-EcoRI restricted plasmid pMP52 to produce plasmid pMP53 (Fig.9A). The 899bp EcoRI-SpeI SFV fragment was cloned into EcoRI-SpeI restricted pMP52 to produce pMP54 (Fig.9A). Plasmid pMP54 was then
20 restricted with SpeI and made blunt-ended with Mung Bean nuclease. The plasmid was then restricted with BglII, dephosphorylated and ligated to the hepatitis delta virus ribozyme linker (SEQ ID nos. 9 and 10), that had been phosphorylated, to produce pMP55 (Fig.
25 9B).

 Plasmid pMP52 was created by ligating a linker (SEQ ID nos:7,8), into the EcoRI site of pUC19 (Fig.10).

 The 1578bp EcoRI-SFV fragment was cloned into
30 the EcoRI site of pUC19, to produce pMP46 (Fig.11A). This plasmid was then restricted with PpuM1 and made

blunt-ended with Mung Bean nuclease. The rabbit β -globin intron II PCR fragment (Fig.1) was made blunt-ended with Mung Bean nuclease, phosphorylated and ligated to the PpuMI restricted pMP46 to produce
5 plasmid pMP70 (Fig.11B).

The 3627bp EcoRI SFV fragment was cloned into the EcoRI site of pUC19 to produce pMP47 (Fig.11A).

Plasmid pCMV3, which contains the CMV promoter, Intron A sequence, BGH poly A sequence and
10 SU40 poly A sequence, was restricted with NdeI and EcoRV. The 3191bp NdeI-EcoRV fragment was isolated and dephosphorylated. The 1321bp NdeI-EcoRV fragment was isolated and restricted with SacI. The NdeI-SacI fragment of 334bp was isolated (Fig.12A). The isolated
15 SacI-EcoRV PCR fragment, containing the 5'-end of SFV was ligated to the previously isolated 334bp NdeI-SacI fragment and the 3191bp NdeI-EcoRV fragment to produce pMP71 (Fig.12A and 12B).

Plasmid pMP53 was then restricted with EcoRI
20 and BamHI and ligated to the isolated and dephosphorylated 2151bp EcoRI fragment from pMP70 (Fig.8A). This ligation was then restricted with EcoRV and the 4057bp EcoRV-EcoRI fragment purified (Fig.8A).

Plasmid pMP47 was restricted with EcoRI and
25 the 3627bp EcoRI fragment isolated and dephosphorylated (Fig.8B). Plasmid pMP55 was then restricted with BglII, dephosphorylated and restricted with EcoRI. The 985bp EcoRI-BglII fragment was isolated and ligated to the previously isolated EcoRI fragment from pMP47
30 (Fig.8B). The ligation reaction was then

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phosphorylated and the 4612bp EcoRI-BglII fragment isolated.

Plasmid pMP71 was restricted with EcoRV and BamHI then dephosphorylated. This fragment was used in a 3-way ligation with the previously isolated 4612bp EcoRI-BglII fragment from pMP47 and pMP55, and the 4057bp EcoRV-EcoRI fragment from pMP53 and pMP70, to produce pMP76 (Figs.8B and 8C).

The 5' end of the SFV replicon was produced by PCR amplification of pSFV1 using primers SFV-5'-3' having the sequence

5'-ATCTATGAGCTCGTTTAGTGAACCGTATGGCGGATGTGTGACATACA-3'

and EcoR-SPE having the sequence

5'-TCCACCTCCAAGGATATCCAAGATGAGTGTG-3' (SEQ ID no: 9 and SEQ ID no: 10 respectively) between the CMV promoter and the 5' end of the SFV replicon. The resulting PCR fragment was restricted with SacI and EcoRV (Fig. 13; SEQ ID no: 11) and the fragment isolated.

SUMMARY OF DISCLOSURE

In summary of this disclosure, the present invention provides a modified alphavirus-based expression vector wherein at least one optimal splice site is introduced to the alphavirus replicon to prevent aberrant splicing of the alphavirus genome; and improve transport of RNA out of the nucleus. Modifications are possible within the scope of the invention.

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CLAIMS

1. An expression vector, comprising a DNA molecule complementary to at least part of an alphavirus RNA genome, which DNA molecule comprises the complement of the complete alphavirus RNA genome regions which are essential for replication of the said alphavirus RNA and further comprises a heterologous DNA sequence capable of expression in a host, said heterologous DNA sequence being inserted into a region of the DNA molecule which is non-essential to replication thereof, and the DNA molecule being placed under transcriptional control of a promoter sequence functional in said host, wherein at least one heterologous splice site is provided in the DNA molecule to prevent aberrant RNA splicing of the alphavirus.
2. The vector of claim 1 wherein said promoter is placed upstream of the 5'-end of the DNA molecule such that the resultant transcript has an authentic 5'-end.
3. The vector of claim 2 wherein said promoter is the cytomegalovirus immediate early promoter.
4. The vector of claim 1 which further comprises an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the DNA molecule.
5. The vector of claim 4 wherein said additional DNA sequence comprises a hepatitis delta ribozyme sequence.
6. The vector of claim 1 wherein the heterologous splice site sequence is provided by the DNA sequence of the rabbit β -globin intron II.
7. The vector of claim 6 wherein the heterologous splice site sequence is inserted into the DNA molecule

at a location which generates perfect splice junctions and restores the function of the SFV replicon when removed.

8. The vector of claim 1 wherein the alphavirus is a
5 Simliki Forest virus.

9. A cloning vector suitable for expression in a host cell of an heterologous DNA sequence, which comprises:
a DNA molecule complementing to at least part of an alphavirus RNA genome, which DNA molecule comprises
10 the complement of the complete alphavirus RNA genome regions and has a cloning site for insertion therein of a heterologous DNA sequence capable of expression in a host cell, said cloning site being located in a region of the DNA molecule which is non-essential to
15 replication thereof;

a promoter sequence functional in said host cell and transcriptionally controlling said DNA molecule, said promoter sequence being placed upstream of the 5'-end of the DNA molecule such that the resultant
20 transcript had an authentic 5' end;

at least one heterologous splice set provided in the complement of the DNA molecule to permit aberrant RNA splicing of one to generate perfect splice junctions in the alphavirus; and

25 an additional DNA sequence at the 3'-end of the DNA molecule to direct proper *in vivo* cleavage at the 3'-end of the reactant RNA molecule.

10. The cloning vector of claim 9 wherein said heterologous splice set is provided by the DNA sequence
30 of the rabbit β -globin intron II.

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11. The cloning vector of claim 9 wherein said additional sequence comprises a hepatitis delta ribozyme sequence.

12. The cloning vector of claim 8 wherein the
5 alphavirus is a Semliki Forest virus.

13. The cloning vector of claim 8 which has the identifying characteristics of plasmid pMP76 shown in Figure 8D.

14. The cloning vector of claim 8 having SEQ ID no:
10 11.

FIG.1

Nucleotide Sequence of the β -globin intron II with the 3' SFV bases

gtgagtttgg ggacccttga ttgttctttc ttttcgcta ttgtaaaatt catgttatat 60
 ggagggggca aagttttcag ggtgttggtt agaattgggaa gatgtccctt gtatcaccat 120
 ggaccctcat gataattttg tttctttcac tttctactct gttagacaacc attgtctcct 180
 cttattttct tttcattttc tgtaactttt tcgttaaaact ttagcttgca ttgtaacga 240
 atttttaaat tcacttttgt ttatttgtca gattgtaagt actttctcta atcaactttt 300
 tttcaaggca atcagggtat attatattgt acttcagcac agttttagag aacaatttgt 360
 ataattaaat gataaggtag aatatattctg catataaatt ctggctggcg tggaatatat 420
 cttattggta gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa 480
 tgatatacac tgtttgagat gaggataaaa tactctgagt ccaaacccggg cccctctgct 540
 aaccatgttc atgccttctt ctttttccta caggtc 576

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FIG.2

Nucleotide Sequence of the β -globin intron II

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gtgagtttgg	ggacccttga	ttgttctttc	tttttcgcta	ttgtaaaatt	catgttatat	60
ggagggggca	aagtttttcag	ggtgttgttt	agaatgggaa	gatgtcccctt	gtatcaccat	120
ggaccctcat	gataattttg	tttcttttcac	tttctactct	gttgacaacc	attgtctcct	180
cttattttct	tttcattttc	tgtaactttt	tcgttaaaact	ttagcttgca	tttgtaacga	240
atthtttaaat	tcacttttgt	ttatttgtca	gattgtaagt	actttctcta	atcacattttt	300
tttcaaggca	atcagggtat	attatatgt	acttcagcac	agttttagag	aacaattggt	360
ataattaaat	gataaggtag	aatatattctg	cataataatt	ctggctggcg	tggaaatat	420
cttattggta	gaaacaacta	catcctggtc	atcatcctgc	ctttctcttt	atggttacaa	480
tgatatacac	tgtttgagat	gaggataaaa	tactctgagt	ccaaaccggg	cccctctgct	540
aaccatgttc	atgccttctt	ctttttccta	cag			573

FIG. 3A

Eco RV-SpeI Fragment of Semliki Forest virus replicon

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atcggcagtg	cgccttccag	gagaatgatg	tctacgcaca	aataccactg	cgtatgccct	60
atgcgcagcg	cagaagaccc	cgaaggctc	gatagtctacg	caaagaaact	ggcagcggcc	120
tccgggaagg	tgctggatag	agagatcgca	ggaataatca	ccgacctgca	gaccgtcatg	180
gctacgccag	acgctgaatc	tcctacctt	tgctgcata	cagacgtcac	gtgtcgtacg	240
gcagccgaag	tggccgtata	ccaggacgtg	tatgctgtac	atgcaccaac	atcgctgtac	300
catcaggcga	tgaagggtgt	cagaacggcg	tattggattg	ggtttgacac	caccccgttt	360
atgtttgacg	cgctagcagg	cgcgtatcca	acctacgcca	caactgggc	cgacgagcag	420
gtgttacagg	ccaggaacat	aggactgtgt	gcagcatcct	tgactgaggg	aagactcggc	480
aaactgtcca	ttctccgcaa	gaagcaattg	aaaccttgcg	acacagtcac	gttctcggta	540
ggatctacat	tgtacactga	gagcagaaag	ctactgagga	gctggcactt	acctccgta	600
ttccacctga	aaggtaaaca	atcctttacc	tgtagggtgcg	ataccatcgt	atcatgtgaa	660
gggtacgtag	ttaagaaaat	cactatgtgc	ccgggcctgt	acggtaaaac	ggtaggggtac	720
gccgtgacgt	atcacgcgga	gggattccta	gtgtgcaaga	ccacagacac	tgtcaaggga	780
gaaagagtct	cattcccctgt	atgcacctac	gtcccctcaa	ccatctgtga	tcaaatgact	840
ggcatactag	cgaccgacgt	cacaccggag	gacgcacaga	agttgttagt	gggattgaat	900
cagaggatag	ttgtgaacgg	aagaacacag	cgaaacacta	acacgatgaa	gaactatctg	960
cttccgattg	tggccgtcgc	atttagcaag	tgggcgaggg	aatacaaggc	agaccttgat	1020
gatgaaaac	ctctgggtgt	ccgagagagg	tcacttactt	gctgctgctt	gtgggcattt	1080
aaaacgagga	agatgcacac	catgtacaag	aaaccagaca	ccagacaat	agtgaagggtg	1140
ccttcagagt	ttaactcgtt	cgtcatcccg	agcctatggt	ctacaggcct	cgcaatccca	1200
gtcagatcac	gcattaaagt	gcttttggcc	aagaagacca	agcgagagtt	aatacctgtt	1260
ctcgacgcgt	cgtcagccag	ggatgctgaa	caagaggaga	aggagaggtt	ggaggcccgag	1320
ctgactagag	aagccttacc	acccctcgtc	cccatcgcgc	cggcggagac	gggagtcgtc	1380
gacgtcgacg	ttgaagaact	agagtatcac	gcagggtgcag	gggtcgtgga	aacacctcgc	1440
agcgcgttga	aagtcaccgc	acagccgaac	gacgtactac	taggaaatta	cgtagtctctg	1500

FIG.3B

tccccgcaga ccgtgctcaa gagctccaag ttggcccccg tgcaccctct agcagagcag 1560
 gtgaaaataa taacacataa cgggagggcc ggcggttacc aggtcgacgg atatgacggc 1620
 agggtcctac taccatgtgg atcggccatt atcggccctg ccggtccctg agtttcaagc tttagacgag 1680
 agcgccacta tgggtgtacaa cgaaggaggag ttcgtcaaca ttcgttcaata ccatattgcc 1740
 gttcacggac cgtcgctgaa caccgacgag caccgacgag gagaactacg agaaagtcag agctgaaaga 1800
 actgacgccg agtacgtgtt agagctaacc cgacgtagat aaaaatgct gaaacaagcag agaggaagcg 1860
 tcgggtttgg tgttgggtggg tggtgggtggg agagcctaac aacccccgt tccatgaatt cgcctacgaa 1920
 gggctgaaga tcaggccgtc gcaagtctgc tattattaag agcctcgtga aagactacag tagtaggagt ctttgggggtt 1980
 ccgggatcag gcaagtctgc gcaagtctgc ccaggaaata gttaacgacg ccaaacacga tctgggtcacc 2040
 agcgggcaaga aggagaactg aggaaaacag tgactccatc ctgctaaacg tgaagaagca ccgcgggaag 2100
 gggacaagta atcctatatg tggacgaggc ttctcgcttgc agtggtgta tgcggagacc ccaagcaatg cgaattcttc 2160
 ctctgttaaac agcttaaggt gaacttcaac agtggtgta tgcggagacc ccaagcaatg cgaattcttc 2220
 aatatgatgc gacgttgcac gacgttgcac cccgtgcaac catcgtgtta acatgcttcc gaggtgggc 2280
 agtatacca gacgttgcac gacgttgcac cccgtgcaac catcgtgtta acatgcttcc gaggtgggc 2340
 ggcaagatgc gcacgaccaa agccaggaga accgtggaca cgaagtcatg acagcagcag catctcaggg 2400
 accaagccca cagtgggact accgtggaca acgcccgtaa atgtactgct gacgcgcaat gggatagggt 2460
 cagtgggact aaaggggtat gagcacgtga atgtactgct ggatagggt ggaagaaga aagaagaaca 2520
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2580
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2640
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2700
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2760
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2820
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2880
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 2940
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 3000
 gagcacgtga ggcatccct gaagaatggc tccagaacaa tccagagatt gaatcagatt gaatcagatt 3060

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FIG.3C

cactgggata acagacctgg tggaaggatg tatggattca atgccgcaac agctgccagg 3120
 ctggaagcta gacatacctt cctgaagggg cagtggcata cgggcaagca ggcagttatc 3180
 gcagaaagaa aaatccaacc gctttctgtg ctggacaatg taattcctat caaccgcagg 3240
 ctgccgcacg ccctgggtggc tgaagtacaag acggttaaag gcagtagggt tgagtggtg 3300
 gtcaataaag taagagggtta ccacgtcctg ctggtgagtg agtacaacct ggctttgcct 3360
 cgacgcaggg tacttggtt gtcaccgctg aatgtcacag gcgccgatag gtgctacgac 3420
 ctaagttag gactgccggc tgacgccggc aggttcgact tggctcttgt gaacattcac 3480
 acggaattca gaatccacca ctaccagcag tgtgtcgacc acgccatgaa gctgcagatg 3540
 ctggggggag atgcgctacg actgctaaaa agccgttgtt cccggcggca tcttgatgag 3600
 tacgccgata aaatcagcga agccgttgtt tcctccttaa gcagaaaagt gcgtctgca 3660
 agagtgttgc gcccggttg tgtcaccagc aatacagaag tgttcttgct gtctctccaac 3720
 tttgacaacg gaaagagacc ctctacgcta cacggccggg gtgtcaccat cctacagagt 3780
 tatgccggag aagccatgca cagggcctgtg agcggcctgtg aaatggccgt cagcctttaa 3840
 gacatagcca cgtgcacaga atgcaaggc cgtggcgaag aacagtcctg tgccggctcgt 3900
 ggggatggcg tatgcaggc gactgaagcg gaaggggacc tcactgagca gcgtagccat 3960
 acaccagtgg gcacaatcaa aacagtcctg gactgaagcg aaacagactg cggaaagagt 4020
 gcgcctaatt tctctgccac ccgccgaagt tgttcagcgg acgccaaggga agtgcacgag 4080
 cgggcagtgg tccacaggag tgttcagcgg aggaagccat agaaatacc agaaatacc 4140
 acagcaatgg acgccaaggga cgtgacgtg tgacatgagg gagagtgcac gctgtactcg 4200
 aagaaatacc aggaagccat cagacttggc gagacttggc gctgtactcg actgacgttg 4260
 gagctgacca ctgacgggtc tggcagagat acgcgctggg catcaacacc tcccaggaca 4320
 tacagtacca gctattgata atatgcctat gattccgatt 4380
 gctattgata atatgcctat gattccgatt 4440
 gctattgata atatgcctat gattccgatt 4500
 gctattgata atatgcctat gattccgatt 4560
 gctattgata atatgcctat gattccgatt 4620

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FIG.3D

gcagaacgga tcgcccgcct taggtcacac caagttaaaa gcatggtggt ttgctcatct 4680
tttcccctcc cgaatatacca tgtagatggg gtgcagaagg taaagtgcga gaaggttctc 4740
ctgttcgacc cgacgggtacc ttcagtgggt acgaggggtt gacttggaat agtatgccgc atctacgacg 4800
gaccactcag atcgggtcgt ataccatgtc agtgacggct agtgacaccc gacgtacacc ctgaacccgc aggcattcgcg 4860
actgccagcg ataccatgtc ctcccatagt cctgaacccc agctgcatac cctgaacccc gtgacatcga ctcgattctac 4920
gagccaatgg ctcccatagt cagatgtgca cccgaagag gcccgaagag gacatgtgca ctcccatagt 4980
gacctggcgg gacctgaagc gaaagccgac actttgacga acgttcctgcg atttacaaca agaggagaa 5040
cctccaccgc cgggcgccga acgttcggcg gacttcgacg ggcagcggac gatgcggtcc agagggagaa 5100
cgggcgccga acgttcggcg gacttcgacg ggcagcggac gatgcggtcc agagggagaa 5160
acgttcggcg gacttcgacg ggcagcggac gatgcggtcc agagggagaa 5220
gacttcgacg ggcagcggac gatgcggtcc agagggagaa 5280
ggcagcggac gatgcggtcc agagggagaa 5340
gatgcggtcc agagggagaa 5400
ttgctgctga aatgcagat aaatgcagat acatgaaagc acgtaggccg tgatcgaaag 5460
aaagtggaga acatgaaagc acgtaggccg tgatcgaaag 5520
acgggagcgg tccctaccg tacctatcca gaaattaccc tggttgacgg gctacccgaa 5580
tacttggaaca aagctccggt ccgtcaccct ttcagaacac aaatgcgaga atgcctgctc 5640
aacgtcacgc ttcaagcgct ataaccactg atgctgctc agaaactgac 5700
cagtgccggtc 5760
cagtgccggtc 5820
gagaaactgc 5880
cgtggagtg 5940
acctatccg 6000
agctgctg 6060
cagattcacg 6120
ggaagaccc 6180

FIG.3E

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aaagtccagg	taattcaagc	agcggagcca	ttggcgaccg	cttacctgtg	cggcatccac	6240
aggaattag	taaggagact	aatgctgtg	ttacgcccta	acgtgcacac	attgtttgat	6300
atgtcggccg	aagactttga	cgcgatactc	gcctctcact	tccaccagg	agaccgggtt	6360
ctagagacgg	acattgcatc	attcgacaaa	agccaggacg	actccttggc	tcttacaggt	6420
ttaatgatcc	tcgaagatct	aggggtggat	cagtacctgc	tggacttgat	cgaggcagcc	6480
tttggggaaa	tatccagctg	tcacctacca	actggcacgc	gcttcaagtt	cggagctatg	6540
atgaaatcgg	gcatgtttct	gactttgttt	attaacactg	ttttgaacat	caccatagca	6600
agcaggggtac	tggagcagag	actcactgac	tccgcctgtg	cggccttcat	cggcgacgac	6660
aacatcgttc	acggagtgat	ctccgacaag	ctgatggcgg	agaggtgcg	gtcgtgggtc	6720
aacatggagg	tgaagatcat	tgacgctgtc	atgggcgaaa	aaccccata	tttttgtggg	6780
ggattcatag	tttttgacag	cgtcacacag	accgcctgcc	gtgtttcaga	ccactttaag	6840
cgcctgttca	agttgggtaa	gccgctaaca	gctgaagaca	agcaggacga	agacaggcga	6900
cgagcactga	gtgacgaggt	tagcaagtgg	ttccggacag	gcttgggggc	cgaactggag	6960
gtggcactaa	catctaggta	tgaggtagag	ggctgcacaa	gtatcctcat	agccatggcc	7020
accttggcga	gggacattaa	ggcgtttaag	aaattgagag	gacctgttat	acacctctac	7080
ggcggtccta	gattgggtgcg	ttaatacaca	gaattctgat	tggatcatag	cgcactatta	7140
taggatccag	atcccgggta	attaattgaa	ttacatccct	acgcacaaagt	tttacggccg	7200
ccggtggcgc	ccgcgcccgg	cggcccgtcc	ttggccgttg	caggccactc	cggtggtctcc	7260
cgtcgtcccc	gacttccagg	cccagcagat	gcagcaactc	atcagcgccg	taaatgcgct	7320
gacaatgaga	cagaacgcaa	ttgctcctgc	taggcctccc	aaaccaaaaga	agaagaagac	7380
aaccaaacca	aagccgaaaa	cgcagcccaa	gaagatcaac	ggaaaaaacgc	agcagcaaaa	7440
gaagaaagac	aagcaagccg	acaagaagaa	gaagaaaacc	ggaaaaaagag	aaagaatgtg	7500
catgaagatt	gaaaatgact	gtatcttcgt	atgcgggctag	ccacagtaac	gtagtgtttc	7560
cagacatgtc	gggcaccgca	ctatcatggg	tgcagaaaaat	ctcgggtggt	ctggggggcct	7620
tcgcaatcgg	cgtatccctg	gtgctgggtg	tggtcacttg	cattgggctc	cgcagataaag	7680
ttagggtagg	caatggcatt	gatatagcaa	gaaaattgaa	aacagaaaaa	gttagggtaa	7740

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FIG.3F

gcaatggcat	ataaccataa	ctgtataact	tgtaacaaag	cgcaacaaga	cctgcgcaat	7800
tggcccccgtg	gtccgcctca	cggaactcg	gggcaactca	tattgacaca	ttaattggca	7860
ataattggaa	gcttacataa	gcttaattcg	acgaataatt	ggatttttat	tttattttgc	7920
aattggtttt	taatatattcc	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	7980
aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa				8010

FIG. 4A

Nucleotide sequence of pSFVlink

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gatggcggat	gtgtgacata	cacgacgcca	aaagattttg	ttccagctcc	tgccacctcc	60
gctacgcgag	agattaaacca	cccacgatgg	ccgccaaaagt	gcatgtttgat	attgaggctg	120
acagcccat	catcaagtct	ttgcagaagg	cattttccgtc	gttcgagggtg	gagtcattgc	180
aggtcacacc	aatgacccat	gcaaatgcc	gagcattttc	gcacctgggt	accaaataga	240
tcgagcagga	gactgacaaa	gacacactca	tcttgatat	cggcagtgcg	ccttccagga	300
gaatgatgtc	tacgcacaaa	taccactgcg	tatgccctat	gcgcagcgca	gaagacccccg	360
aaaggctcga	tagctacgca	aagaaactgg	cagcggcctc	cgggaagggtg	ctggatagag	420
agatcgcagg	aaaaatcacc	gacctgcaga	ccgtcatggc	tacgccagac	gctgaatctc	480
ctacccttttg	cctgcataca	gacgtcacgt	gtcgtacggc	agccgaagtg	gccgtatacc	540
aggacgtgta	tgctgtacat	gcaccaacat	cgctgtacca	tcaggcgatg	aaagggtgtca	600
gaacggcgta	ttggattggg	tttgacacca	cccgttttat	gtttgacgcg	ctagcaggcg	660
cgtatccaac	ctacgccaca	aactgggccg	acgagcaggt	gttacaggcc	aggaacatag	720
gactgtgtgc	agcatccttg	actgagggaa	gactcggcaa	actgtccatt	ctccgcaaga	780
agcaattgaa	accttgcgac	acagtcatgt	tctcggtagg	atctacattg	tacactgaga	840
gcagaaagct	actgaggagc	tggcacttac	cctccgtatt	ccacctga	ggtaaacaat	900
cctttacctg	taggtgcgat	accatcgtat	catgtgaagg	gtacgtagtt	aagaaaatca	960
ctatgtgccc	cggcctgtac	ggtaaaacgg	taggggtacgc	cgtgacgtat	cacgcggagg	1020
gatttcctagt	gtgcaagacc	acagacactg	tcaaaggaga	aagagtctca	ttccctgtat	1080
gcacctacgt	cccctcaacc	atctgtgatc	aatgactgg	catactagcg	accgacgtca	1140
caccggagga	cgcacagaag	ttgttagtgg	gattgaatca	gaggatagtt	gtgaacggaa	1200
gaacacagcg	aaacactaac	acgatgaaga	actatctgct	tccgattgtg	gccgtcgc	1260
ttagcaagtg	ggcgagggaa	tacaaggcag	accttgatga	tgaaaaacct	ctgggtgtcc	1320
gagagaggtc	acttacttgc	tgctgcttgt	gggcatttaa	aacgaggaag	atgcacacca	1380
tgtacaagaa	accagacacc	cagacaatag	tgaagggtgcc	ttcagagttt	aactcgttcg	1440

FIG. 4B

tcatcccgag cctatggtct acaggcctcg caatcccagt cagatcacgc attaatgac 1500
 ttttggccaa gaagaccaag cgagagttaa tacctgttct cgacgcgtcg tcagccaggg 1560
 atgctgaaca agaggagaag gagaggttgg aggcgagct gactagagaa gacttaccac 1620
 ccctcgtccc catcgcccg aggtgcagg gtcgtggaaa cacctcgcag cgcgttgaaa gtcaccgcac 1680
 agtatcacgc aggtgcagg cgtactacta ggaaattacg caccctctag cagagcaggt tagttctgtc 1740
 agccgaacga ggtcccctg ggttaccag gtcgacggat atgacggcag cgcgcagacc cccgcagacc 1800
 gctccaagtt ggtcccctg ggttaccag gtcgacggat atgacggcag cgcgcagacc cccgcagacc 1860
 ggagggcccg ggtcccctg ggttaccag gtcgacggat atgacggcag cgcgcagacc cccgcagacc 1920
 cggccattcc ggtcccctg ggttaccag gtcgacggat atgacggcag cgcgcagacc cccgcagacc 1980
 aaagggagtt cgtcaacagg gaactacgag aaagtacgag aaagtacgag aaagtacgag 2040
 ccgacgagga aaagtacgag aaagtacgag aaagtacgag aaagtacgag aaagtacgag 2100
 acgtagataa aaagtacgag aaagtacgag aaagtacgag aaagtacgag aaagtacgag 2160
 agctaaccac cccccctg gactacagta gactacagta gactacagta gactacagta 2220
 caccataata cctcgtgacc taacgacgtg taacgacgtg taacgacgtg taacgacgtg 2280
 ttattaagag cctcgtgacc taacgacgtg taacgacgtg taacgacgtg taacgacgtg 2340
 aggaatatag taacgacgtg taacgacgtg taacgacgtg taacgacgtg taacgacgtg 2400
 actccatcct gctaaacggg ttccgggtact ttccgggtact ttccgggtact ttccgggtact 2460
 tcgcttgcca ttccgggtact ttccgggtact ttccgggtact ttccgggtact ttccgggtact 2520
 tgggtgttatg cggagacccc caacatctgc caacatctgc caacatctgc caacatctgc 2580
 acttcaacca caacatctgc caacatctgc caacatctgc caacatctgc caacatctgc 2640
 gtccagtcac ggccatctgc ggccatctgc ggccatctgc ggccatctgc ggccatctgc 2700
 cgtgcaacaa acccataatc acccataatc acccataatc acccataatc acccataatc 2760
 tcgtgttaac atgcttccga atgcttccga atgcttccga atgcttccga atgcttccga 2820
 aagtcattgac agcagcagca agcagcagca agcagcagca agcagcagca agcagcagca 2880
 agaaggtgaa tgaaaaatccc tgaaaaatccc tgaaaaatccc tgaaaaatccc tgaaaaatccc 2940
 cgcgcactga ggataggctg gtgtggaaaa cgctggccgg cgatccctgg cgatccctgg 3000

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FIG. 4C

tatcaaacat tccacagggt aactttacgg ccacattgga agaatggcaa gaagaacacg 3060
 acaaaaataat gaaggtgatt gaaggaccgg ctgcgcctgt ggacgcgttc cagaacaaag 3120
 cgaacgtgtg ttgggcgaaa agcctggtgc ctgtcctgga cactgccgga atcagattga 3180
 cagcagagga gtggagcacc ataattacag catttaagga ggacagagct tactctccag 3240
 tgggtggcctt gaatgaaatt tgcaccaagt tgacctggac tgacctggac agacctggtg 3300
 ttcttgcccc gaaggtgtcc ctgtattacg agacaacca ctgggataac ctgggcttcc 3360
 gaaggatgta tggattcaat gccgcaacag ctgccaggct ctgagctaga ggaagctaga 3420
 tgaaggggca gtggcatacg ggcaagcagg cagttatcgc agaaagaaaa agaaagtaaa 3480
 ttctctgtgct ggacaatgta attcctatca accgcaggct accgcacgcc gccgcacgcc 3540
 agtacaagac ggttaaaggc agtagggttg agtggctggt caataaagta agaggggtacc 3600
 acgtccctgct ggtgagttag gtacacaggc tacaacctgg ctctgcctcg acgcagggtc 3660
 caccgctgaa tgtcacaggc gccgatagg gtctttgtga tgcagatgct tggggggagat 3720
 acgccggcag gtctcgacttg gctcgaccac tgctgaagc cttacggata cgcggataaa 3780
 accagcagtg tgtcgaccac ttgatgagag ttgatgagag cgtctgcaag agtgttgcgc 3840
 tgctaaaacc cggcgggcac ctctcttaagc ttcttgctgt tctccaactt tgacaacgga 3900
 ccgttgtttc tacagaagtg ccagatgaat accaagctga gtgccgtgta agagagcaga 3960
 ctacgctaca tgcaccatcc taacgcagct aacgcccgtg gaactgtagg gagcagcaac 4020
 cggccgggtg cggctgtggt atggccgtca gccgtcatcc cccgtcatcc gaattggccg 4080
 tggcgaagaa cagtcattgt aggggaccgc aggggaccgc gaattggccg 4140
 cagtcattgt aggggaccgc aggggaccgc gaattggccg 4200
 ctgaagcggg actgagcagc gctgcagcaa catctactgc agagacaaaa 4260
 acagactgtc gctgcagcaa catctactgc agagacaaaa 4320
 gaagagatag gctgcagcaa catctactgc agagacaaaa 4380
 ctgacgtgac catctactgc agagacaaaa 4440
 ctgacgtgac catctactgc agagacaaaa 4500
 ctgacgtgac catctactgc agagacaaaa 4560

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FIG. 4D

acatgaggac ggctgtggag ttgctcaatg atgacgtgga gctgaccaca gacttggtga 4620
 gagtgcaccc ggacagcagc ctggtgggtc gtaagggcta cagtaccact gacgggtcgc 4680
 tgtactcgtg ctttgaaggt acgaaattca accaggctgc tattgatatg gcagagatac 4740
 tgacgttgtg gcccagactg caagaggcaa acgaacagat atgcctatac gcgctgggcg 4800
 aaacaatgga caacatcaga tccaaatgtc cggatgaacga cgtgattca tcaaacctc 4860
 ccaggacagt gccctgcctg tgccgctacg caatgacagc caatgacagc agaacggatc 4920
 ggtcacacca agttaaaagc atgggtggttt aagtgcgaga gctcatcttt gctcctccc 4980
 tagatggggt gcagaaggtg aagtgcgaga aggttctcct gttcgaccgg gttcgaccctt 5040
 cagtgggttag tccgcggaag tatgccgcat ctacgacgga ctagcagat cactcagat 5100
 gaggggtttga cttggactgg accaccgact cgtcttccac cgtcttccac tgccagcgat 5160
 taccaggttt gcagtcgtgt gacatcgact gacatcgaga gccaatggct cccaatggct 5220
 tgacgggtga cgtacaccct cgtacaccct gaacccgcag gcatcgcgga cctggcggca 5280
 ctgaaccgcg agaccatgtg gacctcgaga gacctcgaga accgatctc tccaccgcg 5340
 ctgcatacct tgcctcccgc gctcctcccgc gagggtggtg gacctggtgc ggcgcgaga 5400
 ctgcccgaag gactgcgttt aggaacaagc tgcctttgac gacctttgac gttcggcgac 5460
 acgagggtcga tgcgttggtc tccgggatta ctttcggaga ctttcggaga cttcgacgac 5520
 taggcccgcg ggggtgcata ggggtgcata attttctcct cggacactgg cagcggacat 5580
 aatccgttag gcagcacaat ctccagtgcg cacaactgga cactgactgg gaggagaaa 5640
 tgtacccgcc aaaattggat agtcgatacc agtcgcaa agtcgcaa agtgagaaac 5700
 acccatcgga ggctaataag agtcgatacc agtcgcaa agtcgcaa agtgagaaac 5760
 cgggtggtga caggctcaca tcggggggcca gattgtacac gattgtacac gggagcggac 5820
 tacciaacata cgcggttcgg taccctccgc ccgtgtactc ccgtgtactc cctaccgtg 5880
 tctcaagccc cgatgtagca atcgacgct atcgacgata gcaacgaata cctatccaga 5940
 cagtggcgtc gtaccagata gtaccagata acagatgaat acgacgcata cttggacatg 6000
 cggatagttg cttggacaga gcgacattct gcccggcgaa gctccggtgc tactccgaaac 6060
 atcatgcgtg ccaccagccg actgtacgca gtgcccgtccc gtcacccttt cagaacacac 6120

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FIG. 4E

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tacagaacgt	gctagcggcc	gccaccaaga	gaaactgcaa	cgtcacgcaa	atgcgagaac	6180
taccacccat	ggactcggca	gtgttcaacg	tggagtgctt	caagcgctat	gcctgctccg	6240
gagaatatg	ggaagaatat	gctaacaac	ctatccgat	aaccactgag	aacatcacta	6300
cctatgtgac	caaattgaaa	ggcccgaag	ctgctgcctt	gttcgctaag	accacaact	6360
tggttccgct	gcaggaggtt	cccatggaca	gattcacggt	cgacatgaaa	cgagatgtca	6420
aagtcactcc	aggacgaaa	cacacagagg	aaagacccaa	agtcaggta	attcaagcag	6480
cggagccatt	ggcgaccgct	tacctgtcg	gcatccacag	ggaattagta	aggagactaa	6540
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cgatcatcgc	ctctcacttc	caccagagg	accggttct	agagacggac	attgcatcat	6660
tcgacaaaag	ccaggacgac	tccttggctc	ttacaggttt	aatgatcctc	gaagatctag	6720
gggtggatca	gtacctgctg	gacttgcacg	aggcagcctt	tggggaata	tccagctgtc	6780
acctaccaac	tggcacgcgc	ttcaagttcg	gagctatgat	gaaatcgggc	atgtttctga	6840
ctttgtttat	taacactgtt	ttgaacatca	ccatagcaag	cagggtactg	gagcagagac	6900
tcactgactc	cgcctgtgcg	gccttcatcg	gcgacgacaa	catcgttcac	ggagtgatct	6960
ccgacaagct	gatggcggag	aggtgcgcgt	cgtgggtcaa	catggagggtg	aagatcatctg	7020
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tcacacagac	cgcctgccgt	gtttcagacc	cacttaagcg	cctgttcaag	ttgggtaagc	7140
cgctaacagc	tgaagacaag	caggacgaag	acaggcgacg	agcactgagt	gacgaggtta	7200
gcaagtgggt	ccggacaggc	ttggggggccg	aactggagggt	ggcactaaca	tctagggtatg	7260
aggtagaggg	ctgcaaaagt	atcctcatag	ccatggccac	cttggcgagg	gacatctaagg	7320
cgtttaagaa	atttgagagg	cctgttatat	acctctacgg	cggtcctaga	ttggtgcgtt	7380
aatacacaga	attctgattg	gatcatagcg	cactattata	ggatccagat	cccgggtaat	7440
taattgaatt	acatccctac	gcaaacgttt	tacggccgcc	ggtggcgccc	gcgcccggcg	7500
gcccgtcctt	ggccgttgca	ggccactccg	gtggctcccg	tcgtccccga	cttccaggcc	7560
cagcagatgc	agcaactcat	cagcgccgta	aatgcgctga	caatgagaca	gaacgcaatt	7620
gctcctgcta	ggcctcccaa	accaagaag	aagaagacaa	ccaaaccaa	gccgaaaaacg	7680

FIG. 4F

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cagccaaga	agatcaacgg	aaaaacgcag	cagcaaaaga	agaaagacaa	gcaagccgac	7740
aagaagaaga	agaaacccgg	aaaaagagaa	agaatgtgca	tgaagattga	aatgactgt	7800
atcttcgtat	gcggctagcc	acagtaacgt	agtgtttcca	gacatgtcgg	gcaccgcact	7860
atcatgggtg	cagaaaatct	cgggtggtct	gggggccttc	gcaatcggcg	ctatcctggt	7920
gctgggttg	gtcacttgca	ttgggctccg	cagataagtt	agggtaggca	atggcattga	7980
tatagcaaga	aaattgaaaa	cagaaaaagt	tagggtaagc	aatggcatat	aaccataact	8040
gtataacttg	taacaaagcg	caacaagacc	tgcgcaattg	gccccgtggt	ccgcctcacg	8100
gaaactcggg	gcaactcata	ttgacacatt	aattggcaat	aattgggaagc	ttacataaagc	8160
ttaattcgac	gaataaattgg	atttttattt	tattttgcaa	ttggtttta	atatttccaa	8220
aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	8280
aaaaaaaaact	agtctgcatt	aatgaaatcgg	ccaacgcgcg	gggagaggcg	gtttgcgtat	8340
tgggcgcctct	tccgcttcct	cgctcactga	ctcgctgcgc	tcggtcgttc	ggctgcggcg	8400
agcggatatca	gctcactcaa	aggcggtaat	acggttatcc	acagaatcag	gggataacgc	8460
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cctcgtgcgc	tctcctgttc	cgaccctgcc	gcttaccgga	tacctgttccg	cctttctccc	8700
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cgttcgctcc	aagctgggct	gtgtgcacga	acccccgtt	cagcccgacc	gctgcgcctt	8820
atccgggtaac	tatcgtcttg	agtccaaccc	ggtaagacac	gacttatcgc	cactggcagc	8880
agccactgggt	aacaggatta	gcagagcgag	gtatgtaggc	ggtgctacag	agttcttgaa	8940
gtgggtggcct	aactacggct	acactagaag	gacagtattt	ggtatctgcg	ctctgctgaa	9000
gccagttacc	ttcgggaaaa	gagttggtag	ctcttgatcc	ggcaaaacaaa	ccaccgctgg	9060
tagcgggtggt	ttttttgttt	gcaagcagca	gattacgcgc	agaaaaaaaag	gatctcaaga	9120
agatcctttg	atcttttcta	cggggtctga	cgctcagtg	aacgaaaact	cacgttaagg	9180
gattttggtc	atgagattat	caaaaaggat	cttcacctag	atccttttaa	attaaaaatg	9240

FIG. 4G

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aagttttaaa	tcaatctaaa	gtatatatga	gtaaatctgg	tctgacagtt	accaatgctt	9300
aatcagtgag	gcacctatct	cagcgatctg	tctatttcgt	tcatccatag	ttgcctgact	9360
ccccgtcgtg	tagataacta	cgatacggga	gggcttacca	tctggcccca	gtgctgcaat	9420
gataccgcga	gaccacgct	caccggctcc	agatttatca	gcaataaacc	agccagccgg	9480
aagggccgag	cgagaaagtg	gtcctgcaac	tttatccgcc	tccatccagt	ctattaattg	9540
ttgccgggaa	gctagagtaa	gtagttcgcc	agttaatagt	ttgcgcaacg	ttgttgccat	9600
tgctacaggc	atcgtggtgt	cacgctcgtc	gtttgggtatg	gcttcattca	gctccggttc	9660
ccaacgatca	aggcgagtta	catgatcccc	catgttgtgc	aaaaaagcgg	ttagctcctt	9720
cggtcctccg	atcgttgtca	gaagtaagtt	ggccgcagtg	ttatcactca	tggttatggc	9780
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gtactcaacc	aagtcattct	gagaatagtg	tatgcggcga	ccgagttgct	cttgcccggc	9900
gtcaatatcgg	gataataccg	cgccacatag	cagaacttta	aaagtgtctca	tcattggaaa	9960
acgttctttcg	gggcgaaaaac	tctcaaggat	cttaccgctg	ttgagatcca	gttcgatgta	10020
accactcgt	gcacccaact	gatcttcagc	atcttttact	ttcaccagcg	tttctgggtg	10080
agcaaaaaaca	ggaaggcaaa	atgccgcaaa	aaagggaata	agggcgacac	ggaaatgttg	10140
aatactcata	ctcttccttt	ttcaatatata	ttgaagcatt	tatcagggtt	attgtctcat	10200
gagcggatac	atattttgaat	gtattttagaa	aaataaacia	ataggggttc	cgcgcacatt	10260
tccccgaaaa	gtgccacctg	acgtctaaga	aaccattatt	atcatgacat	taacctataa	10320
aaataggcgt	atcacgaggc	cctttcgtct	cgcgcgtttc	ggtgatgacg	gtgaaaaacct	10380
ctgacacatg	cagctcccgg	agacgggtcac	agcttctgtc	taagcggatg	ccgggagcag	10440
acaagcccgt	cagggcgcgt	cagcgggtgt	tggcgggtgt	cggggctggc	ttaaactatgc	10500
ggcatcagag	cagatttgtac	tgagagtgca	ccatatacgac	gctctccctt	atgcgactcc	10560
tgcattagga	agcagccccag	tactaggttg	aggccgttga	gcaccgccgc	cgcaagggaat	10620
ggtgcatgca	aggagatggc	gcccacaacagt	cccccggccca	cggggcctgc	caccataccc	10680
acgccgaaac	aagcgctcat	gagcccgaag	tggcgagccc	gatcttcccc	atcgggtgatg	10740
tcggcgatat	aggcgccagg	aaccgcacct	gtggcgccgg	tgatgccggc	cacgatgcgt	10800

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FIG. 4H

ccggcgtaga	ggatctggct	agcgatgacc	ctgctgattg	gttcgctgac	catttccggg	10860
gtgcggaacg	gcgttaccag	aaactcagaa	ggttcgtcca	accaaaccca	ctctgacggc	10920
agtttacgag	agagatgata	gggtctgctt	cagtaagcca	gatgctacac	aattaggctt	10980
gtacatatgtg	tcgttagaac	gcggctacaa	ttaatacata	accttatgta	tcatacacat	11040
acgatttagg	tgacactata					11060

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Construction of pSFVlink

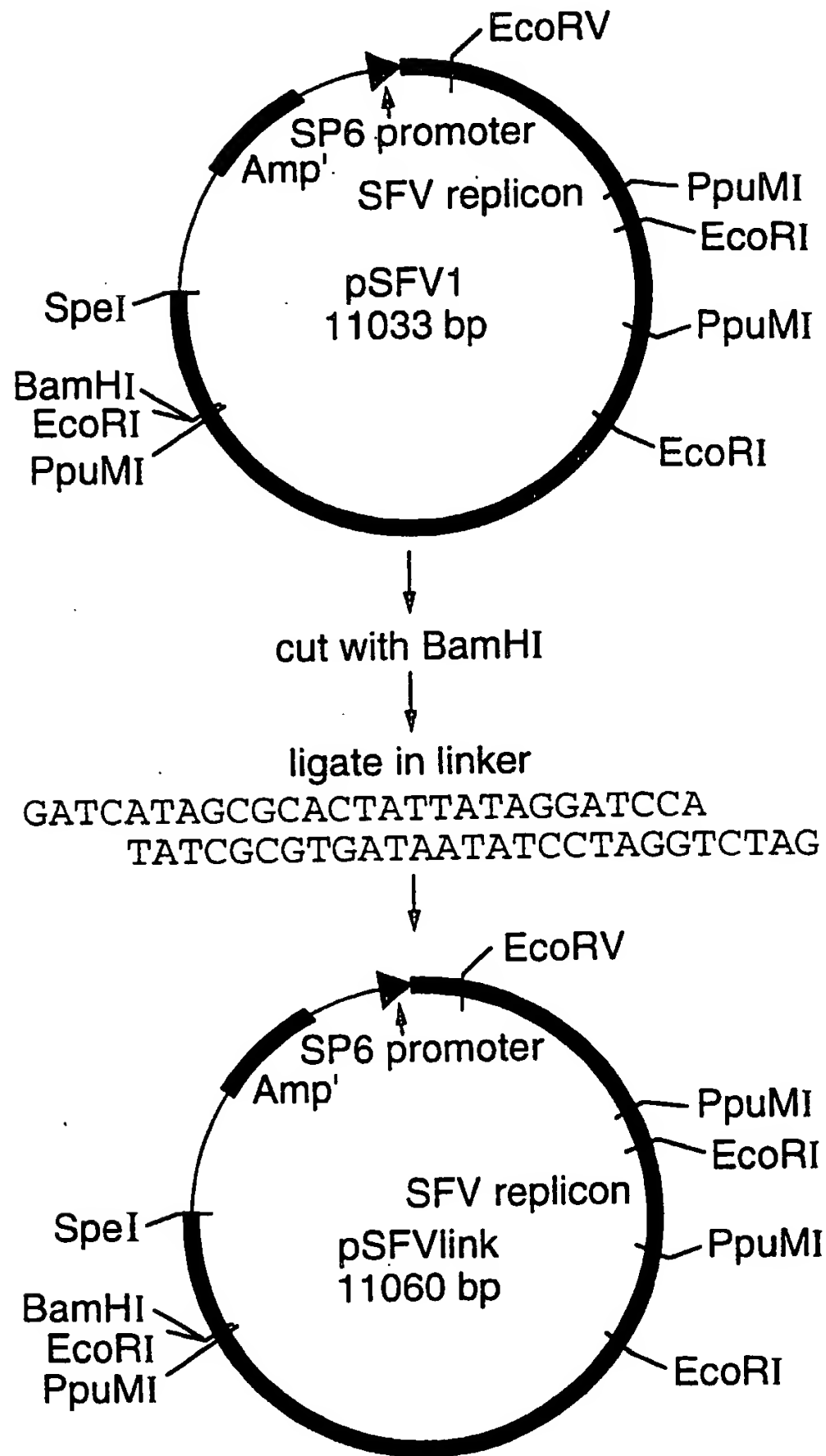


FIG.5

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FIG. 6A

Nucleotide Sequence of pMP76

attggctatt	ggccattgca	tacgttgtat	ctatatcata	atatgtacat	ttatatggc	60
tcatgtccaa	tatgaccgcc	atgttgacat	tgattattga	ctagttatta	atagtaatca	120
attacggggt	cattagttca	tagcccatat	atggagttcc	gcgttacata	acttacggta	180
aatggcccg	ctcgtgaccg	cccaacgacc	ccgcccatt	gacgtcaata	atgacgtatg	240
ttcccatagt	aacgccaata	gggactttcc	attgacgtca	atgggtggag	tatttacggt	300
aaactgcca	cttggcagta	catcaagtgt	atcatatgcc	aagtcggccc	cctattgacg	360
tcaatgacgg	taaatggccc	gcctggcatt	atgcccagta	catgacctta	cgggactttc	420
ctacttggca	gtacatctac	gtattagtca	tcgctattac	catgggtgatg	cggttttggc	480
agtacacca	tgggcgtgga	tagcggtttg	actcacgggg	atttccaagt	ctccaccca	540
ttgacgtcaa	tgggagtttg	ttttggcacc	aaaatcaacg	ggactttcca	aatgtcgtta	600
ataaccccg	cccgttgacg	caaatggcg	gtaggcgtgt	acgggtgggag	gtctatataa	660
gcagagctcg	tttagtgac	cgtatggcgg	atgtgtgaca	tacacgacgc	caaaagattt	720
tgttccagct	cctgccacct	ccgctacgcg	agagattaac	caccacgat	ggccgcca	780
gtgcatgttg	atattgaggc	tgacagccca	ttcatcaagt	ctttgcagaa	ggcatttccg	840
tcgttcgagg	tggagtcatt	gcaggtcaca	ccaatgacc	atgcaaatgc	cagagcattt	900
tcgcacctgg	ctaccaaat	gacgagcag	gagactgaca	aagacacact	catcttggat	960
atcgggcagt	cgccttccag	gagaatgatg	tctacgcaca	aataccactg	cgtatgccct	1020
atgcgcagcg	cagaagaccc	cgaaaggctc	gatagctacg	caaagaaact	ggcagcggcc	1080
tccgggaagg	tgctggatag	agagatcgca	ggaaaaatca	ccgacctgca	gaccgtcatg	1140
gctacgccag	acgctgaatc	tcctaccctt	tgcctgcata	cagacgtcac	gtgtcgtacg	1200
gcagccgaag	tggccgtata	ccaggacgtg	tatgctgtac	atgcaccaac	atcgtgtac	1260
catcaggcga	tgaagggtgt	cagaacggcg	tattggattg	ggtttgacac	caccccgttt	1320
atgtttgacg	cgctagcagg	cgcgtatcca	acctacgcca	caactgggc	cgacgagcag	1380

FIG. 6B

gtgttacagg ccaggaacat aggactgtgt gcagcatcct tgactgaggg aagactcggc 1440
 aaactgtcca ttctccgcaa gaagcaattg aaaccttgcg acacagtcac gtctctcggta 1500
 ggatctacat tgtacactga gacagaaaag ctactgagga gctggcactt accctccgta 1560
 ttccacctga aaggtaaaac atcctttacc cactatgtgc cccggcctgt acggtaaagac 1620
 gggtagctag ttaagaaaat atcacgcgga gggattccta gtgtgcaaga ccacagacac 1680
 gccgtgacgt atcacgcgga cattccctgt atgcacctac gtcccctcaa ccactctgtga 1740
 gaaagagtct cgaccgacgt cgaccgacgt cagaccggag cacaccacaga gacgcacaga 1800
 ggcatactag cagaccgacgt ttgtgaacgg atttagcaag cagagagagg cctcttgagg 1860
 cagaggatag ctcccgattg tggccgtcgc ctctgggtgt cgtcatcccg agcctatggg 1920
 ctcccgattg gatgaaaaac ctctgggtgt cgtcatcccg agcctatggg 1980
 gatgaaaaac ctctgggtgt cgtcatcccg agcctatggg 2040
 aaacgagga agatgcacac ttaactcgtt gcatcgaagt gctgctgctt gctgctgctt 2100
 ccttcagagt ttaactcgtt gcatcgaagt gctgctgctt gctgctgctt gctgctgctt 2160
 gtcagatcac gtcagccag cgtcagccag aagccttacc agagagaggc agcagagagt 2220
 ctgactagag aagccttacc ttgaagaact agagtatcac gacgtactac taggaaatta 2280
 gacgtcgacg aagtcaccgc ccgtgctcaa gagctccaag ggcggttacc aggtcgacgg 2340
 agcgcgttga tccccgcaga gtgaaaataa taacacataa taccatgtgg atcgggccatt 2400
 tccccgcaga gtgaaaataa taacacataa taccatgtgg atcgggccatt 2460
 gtgaaaataa taacacataa taccatgtgg atcgggccatt 2520
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 actgacgccc agtacgtgtt tgttggtggg tccatgaatt 2760
 tccgggtttgg gggctgaaga tcaggccgtc tagtaggagt 2820
 gggctgaaga tccgggtttgg gggctgaaga tccgggtttgg 2880
 gggctgaaga tccgggtttgg gggctgaaga tccgggtttgg 2940

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FIG. 6C

ccgggatcag gcaagtctgc tattattaag agcctcgtga ccaaacacga tctggtcacc 3000
 agcgggcaaga aggagaactg ccaggaaata gttaacgacg tgaagaagca ccgcgggaag 3060
 gggacaagta gggaaaaacag tgactccatc ctgctaaacg ctgtcgtcg ggtgtcgtcg 3120
 atcctatatg tggacgaggc tttcgcttgc agtgggtgtta tgcggagacc ccaagcaatg cggattcttc 3180
 ctgtttaac ctcgagagcaa agcttaaggt gaacttcaac gactgaagt atgtcataaa 3240
 aatatgatgc gacgttgcac ggtccagtc ggtcccatct cactacgga 3300
 agtatatcca gacgttgcac ggtccagtc acggccatct tgctacgtt gactacgga 3360
 ggcaagatgc gcacgaccaa cccgtgcaac aaacccataa tcatagacac cacaggacag 3420
 accaagccca agccaggaga catcgtgtta acatgcttcc gaggctgggc cctcacccgc 3480
 cagtgggact accgtggaca cgaagtcagt gcagaaggtg aatgaaaac acagcagcag 3540
 aaaggggtat acgccgtaag gcagaaggtg aatgaaaac acagcagcag 3600
 gagcacgtga atgtactgct gacgcgcact gaggttgagg acccttgatt 3660
 ggcgatccct ggattaaagt gagtttgggg acccttgatt acccttgatt 3720
 gtaaaattca tggtatatgg agggggcaaa gttttcaggg tggtgtttag aatgggaaga 3780
 tgtcccttgt atcaccatgg accctcatga taattttgtt taacttttctc 3840
 tgacaaccat tgttccctct ttttaaatc acttttgttt atttgtcaga 3900
 agcttgcat tttctctaat caattgttat taaggtagaa taaggtagaa 3960
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 ttctctttat aaaccgggcc agggtaactt accggctgctg gccttcttct 4140
 aacattccac ataataagg tgattgaagg cgttccagaa cgttccagaa 4200
 ataataagg gtgtgttggg cgaaaagcct gagcagcag 4260
 gagagtgga gcaccataat tacagcattt aaggaggaca gagcttactc 4320
 4380
 4440
 4500

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FIG. 6D

gccttgaatg aaatttgcac caagtactat ggagttgacc tggacagtgg cctgttttct 4560
 gccccgaagg tgtccctgta ttacgagaac aacctggg ataacagacc tggtggaagg 4620
 atgtatggat tcaatgccgc aacagctgcc aggctggaag ctagacatac ctccctgaag 4680
 gggcagtgcc atacgggcaa atcgccagtt atcgagaaa gaaaaatcca accgctttct 4740
 gtgctggaca atgtaattcc tatcaaccgc agctgccgc acgccctggc ggctgagtac 4800
 aagacgggta aaggcagtag ggttgagtgg ctggtcaata aagtaagagg gtaccacgtc 4860
 ctgctgggtga gtgagtacaa cctggctttg cctcgacgca ggtcacttg gttgtcacccg 4920
 ctgaatgtca caggcgccga taggtgctac gacctaaagt taggactgcc ggctgacgcc 4980
 ggcagggttcg acttgggtctt tgtgaacatt cacacggaat tcagaatcca cactaccag 5040
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 gtttccctcct taagcagaaa gttctcgtct gctgttctcc aactttgaca acggaagag 5220
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 ctacaccaga tgaataccaa gctgagtgcc agttaaagaga gcagacatag ccacgtgcac 5340
 ggtgtgtcac catcctacag cagctaacgc ccgtggaact taaggaggca gtaagggatg 5400
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 aagaaatggc atgtgcggct accgcgaatt gcagcgtagc accgcgaatt gtagcgccta 5520
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 gataggctgc gtgaccatct tggagtgtgct caatgtatgac caatgtatgac caatgtatgac 5760
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 aggacggctg cagcctgggt aaggtacgaa attcaaccag gctgctattg cagatatgcc 5880
 caccgggaca tcgtactttg gactgcaaga gactgcaaga gactgcaaga gactgcaaga 5940
 tcgtactttg gactgcaaga gactgcaaga gactgcaaga gactgcaaga gactgcaaga 6000
 ttgtggccca gactgcaaga gactgcaaga gactgcaaga gactgcaaga gactgcaaga 6060

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FIG. 6E

atggacaaca	tcagatccaa	atgtccggtg	aacgattccg	attcatcaac	acctcccagg	6120
acagtgcctt	gcctgtgccg	ctacgcaatg	acagcagaac	ggatcgcccg	ccttaggtca	6180
caccaagtta	aaagcatggt	ggtttgctca	tcttttccc	tccgaaata	ccatgtagat	6240
ggggtgcaga	aggtaagt	cgagaagggt	ctcctgttcg	acccgacggt	accttcagtg	6300
gttagtcgc	ggaagtatgc	cgcatctacg	acggaccact	cagatcggtc	gttacgaggg	6360
tttgacttgg	actggaccac	cgactcgtct	tccactgcca	gcgataccat	gtcgctaccc	6420
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gctgacgtac	accctgaacc	cgaggcatc	gcggaccctgg	cggcagatgt	gcaccctgaa	6540
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tcggagggcta	ataagagtcg	ataccagtct	cgcaaatggtg	agaacatgaa	agccacggtg	7020
gtggacaggc	tcacatcggg	ggccagattg	tacacgggag	cgacgtagg	ccgcatacca	7080
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aacgtgctag	cggccgccac	caagagaaac	tgcaacgtca	cgcaaatgcg	agaactaccc	7440
accatggact	cggcagtgtt	caacgtggag	tgcttcaagc	gctatgcctg	ctccggagaa	7500
tattgggaag	aatatgctaa	acaacctatc	cggataacca	ctgagaacat	cactacctat	7560
gtgaccaaat	tgaaaggccc	gaaagctgct	gccttgttcg	ctaagaccca	caacttgggt	7620

FIG. 6F

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ccgctgcagg	aggttcccat	ggacagattc	acggtcgaca	tgaaacgaga	tgtcaaagtc	7680
actccaggga	cgaaacacac	agaggaaaga	cccaagtcc	aggtaattca	agcagcggag	7740
ccattggcga	ccgcttacct	gtgcggcatc	cacagggaat	tagtaaggag	actaatgct	7800
gtgttacgcc	ctaacgtgca	cacattgttt	gatatgtcgg	cgaagactt	tgacgcgac	7860
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aaaagccagg	acgactcctt	ggctcttaca	ggtttaatga	tcctcgaaga	tctaggggtg	7980
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ccaactggca	cgcgcttcaa	gttcggagct	atgatgaat	cgggcatgtt	tctgactttg	8100
tttattaaaca	ctgttttgaa	catcaccata	gcaagcaggg	tactggagca	gagactcact	8160
gactccgcct	gtgcggcctt	catcggcgac	gacaacatcg	ttcacggagt	gatctccgac	8220
aagctgatgg	cggagaggtg	cgcgtcgtgg	gtcaacatgg	aggtgaagat	cattgacgct	8280
gtcatgggcg	aaaaacccc	atattttgt	gggggattca	tagtttttga	cagcgtcaca	8340
cagaccgcct	gccgtgttct	agaccactt	aagcgcctgt	tcaagtggg	taagccgcta	8400
acagctgaag	acaagcagga	cgaagacagg	cgcgagcac	tgagtgcga	ggttagcaag	8460
tggttccgga	caggcttggg	ggccgaactg	gaggtggcac	taacatctag	gtatgaggta	8520
gagggctgca	aaagtatcct	catagccatg	gccaccttgg	cgagggacat	taaggcgttt	8580
aagaaattga	gaggacctgt	tatacacctc	tacggcggtc	ctagattggt	gcgttaatac	8640
acagaattct	gattggatca	tagcgcata	ttataggatc	cagatcccgg	gtaattaat	8700
gaattacatc	cctacgcaaa	cgttttacgg	ccgccggtgg	cgcccgcgcc	cggcggccc	8760
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gatgcagcaa	ctcatcagcg	ccgtaaatgc	gctgacaatg	agacagaacg	caattgctcc	8880
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caagaagatc	aacggaaaaa	cgcagcagca	aaagaagaaa	gacaagcaag	ccgacaagaa	9000
gaagaagaaa	cccggaaaaa	gagaaagaat	gtgcatgaag	attgaaaaatg	actgtatctt	9060
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gggtgcagaa	aatctcgggt	ggtctggggg	ccttcgcaat	cggcgctatc	ctggtgctgg	9180

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FIG. 6G

ttgtggtcac	ttgcattggg	ctccgcagat	aagttagggt	aggcaatggc	attgatatag	9240
caagaaaaatt	gaaaacagaa	aaagttaggg	taagcaatgg	catataacca	taactgtata	9300
acttgtaaca	aagcgcaaca	agacctgcgc	aattggcccc	gtggtccgcc	tcacggaaac	9360
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aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	aaaaaaaaaa	9540
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tgttgtttgc	ccctcccccg	tgctttcctt	gacctggaa	ggtgccactc	ccactgtcct	9720
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gcggcgagcg	gtatcagctc	actcaaggc	ggtaatacgg	ttatccacag	aatcagggga	10320
taacgcagga	aagaacatgt	gagcaaaagg	ccagcaaaag	gccagggaacc	gtaaaaaggc	10380
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tctcccttcg	ggaagcgtgg	cgctttctca	tagctcacgc	tgtaggatc	tcagttcgg	10620
gtaggtcggt	cgctccaagc	tgggctgtgt	gcacgaaccc	cccgttcagc	ccgaccgctg	10680
cgccttatcc	ggtaactatc	gtcttgagtc	caaccgggt	agacacgact	tatcgccact	10740

FIG. 6H

ggcagcagcc actggtaaca ggattagcag agcgaggatat gtaggcggtg ctacagagtt 10800
 cttgaagtgg tggcctaact acggctacac tagaaggaca gtatttggta tctgcgctct 10860
 gctgaagcca gttaccttcg gaaaaagagt tggtagctct tgatccggca aacaaaccac 10920
 cgctggtagc ggtggttttt ttgtttgcaa gcagcagatt acgcgcagaa aaaaaggatc 10980
 tcaagaagat cctttgatct ttctacggg gtctgacgct gtctggaacg aaactcacg 11040
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 aaaatgaagt tttaaatcaa tctaaagtat atattagtaa acttgggtctg acagttacca 11160
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 ctgactccgg gggggggggg cgctgaggtc tgcctcgtga agaagggtgtt gctgactcat 11280
 accaggcccg aatcgcccca tcatccagcc agaaagttag ggagccacgg ttgatgagag 11340
 ctttgttgta ggtggaccag ttggtgattt tgaacttttg ctctgccacg gaacgggtctg 11400
 cgttgtcggg aagatgcgtg atctgatcct tcaactcagc tggtacaacc aattaacca 11460
 aaagccgccg tcccgtaag ttagcgtaat gcctgcccag gcttatacca tttattcaac 11520
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 gttccatagg atggcaagat cctggtatcg gtctgcgatt gtctgcgatt cccactcgtc caacatcaat 11700
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 aggccagcca ttacgctcgt catcaaaaatc gaaatacgcg atcgctgtta' aaaggacaat tacaacacag 11880
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 cagaaacaac tctggcgcat cccgagccc atttataccc atataaatca gcattccatgt 12240
 cccgacatta tcgagagccc atataaatca gcattccatgt 12300

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FIG. 6I

```

tcgcggcctc gagcaagacg tttcccgttg aatatggctc ataacacccc ttgtattact 12360
gtttatgtaa gcagacagtt ttattgttca tgatgataata tttttatctt gtgcaatgta 12420
acatcagaga ttttgagaca caacgtggct ttccccccc cccccgagct tgat 12474

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CMV promoter 1 - 682
SFV replicon (before intron) 684 - 3678
Rabbit (-globin intron II 3679 - 4251
SFV replicon (after intron) 4252 - 9543
Hepatitis Delta virus ribozyme (antigenomic) 9544 - 9628
Kanamycin Gene 12342 - 11503
BamHI site for insertion of heterologous inserts 8677

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Subcloning of the SFV replicon

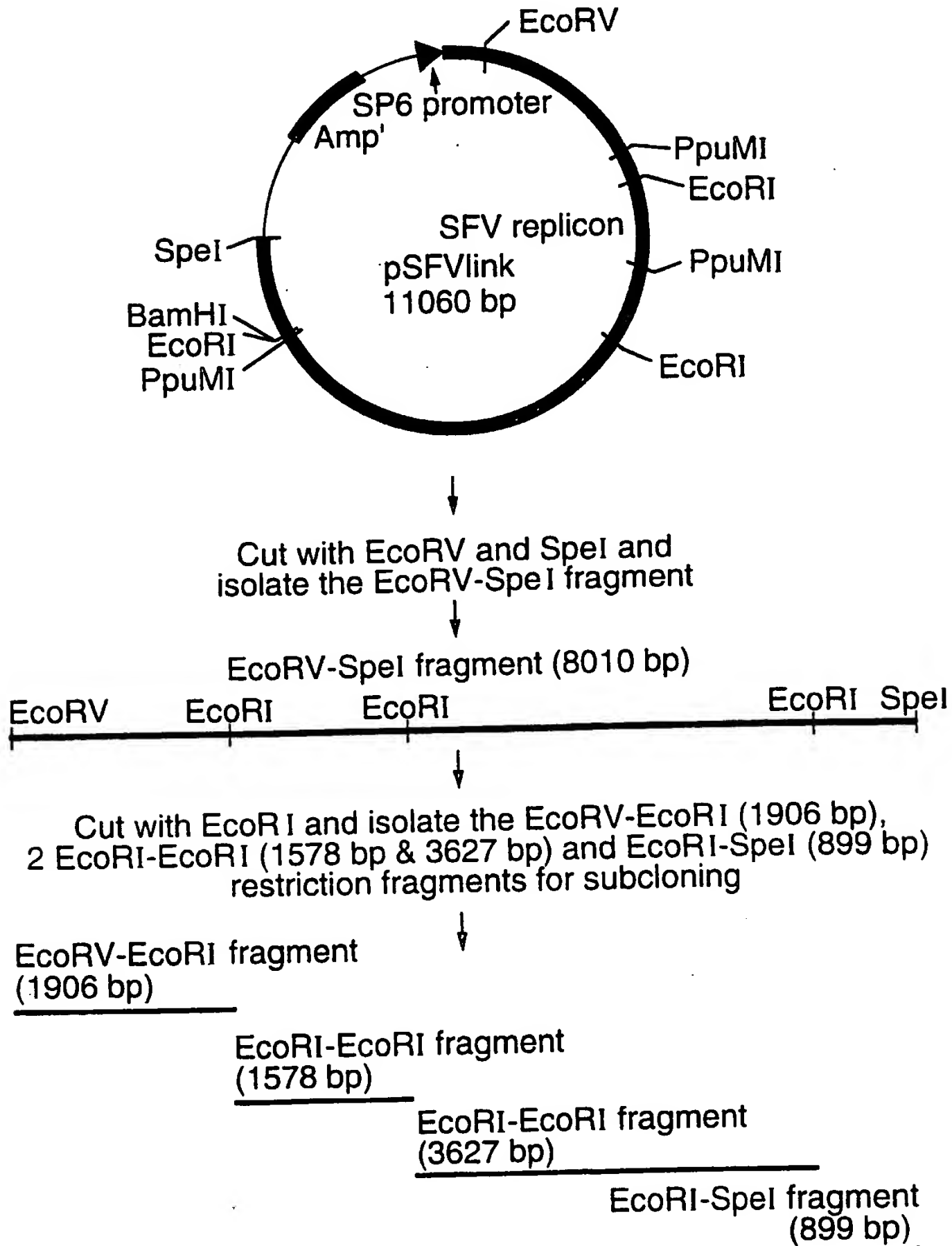


FIG.7

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Construction of pMP76

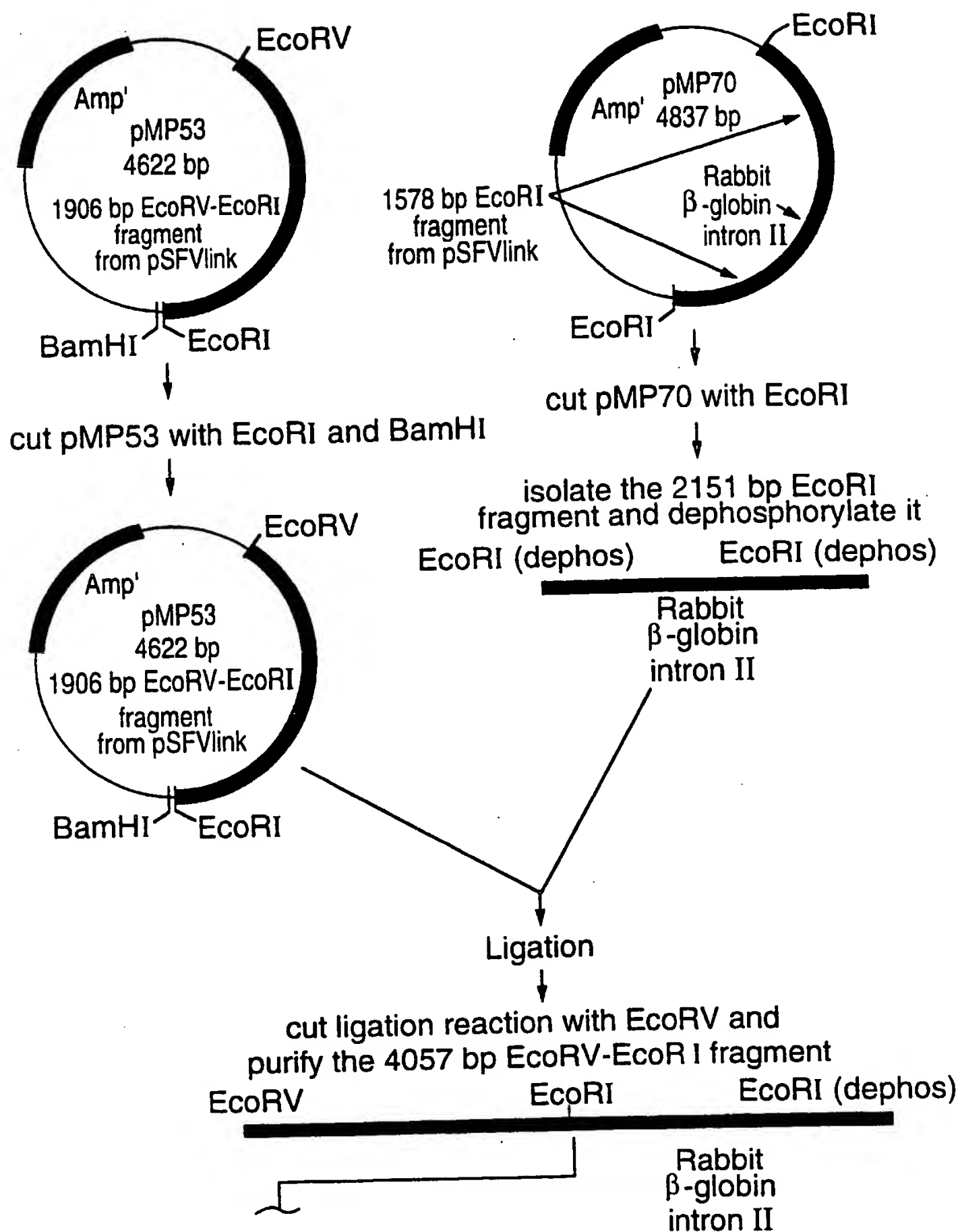


FIG.8A

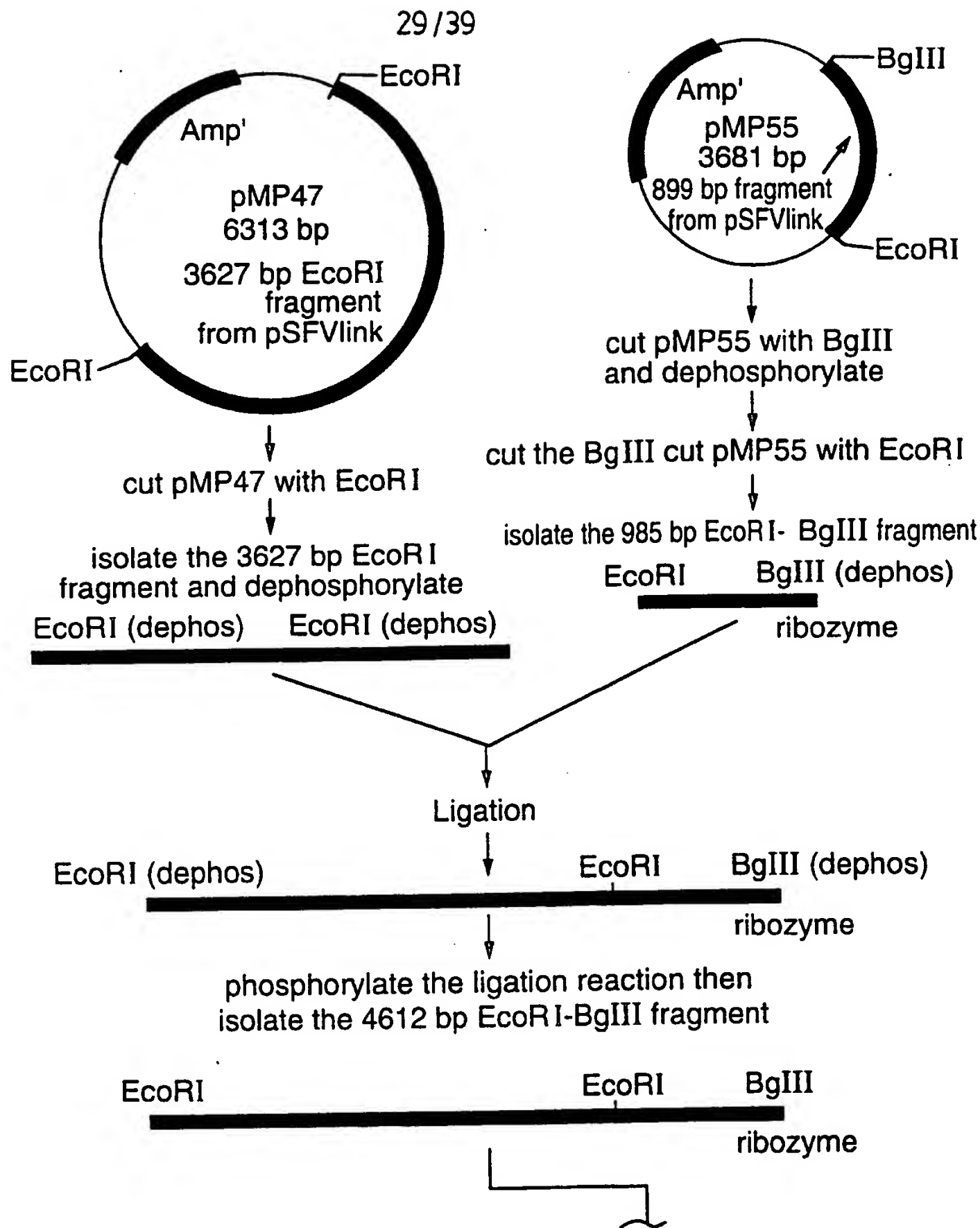
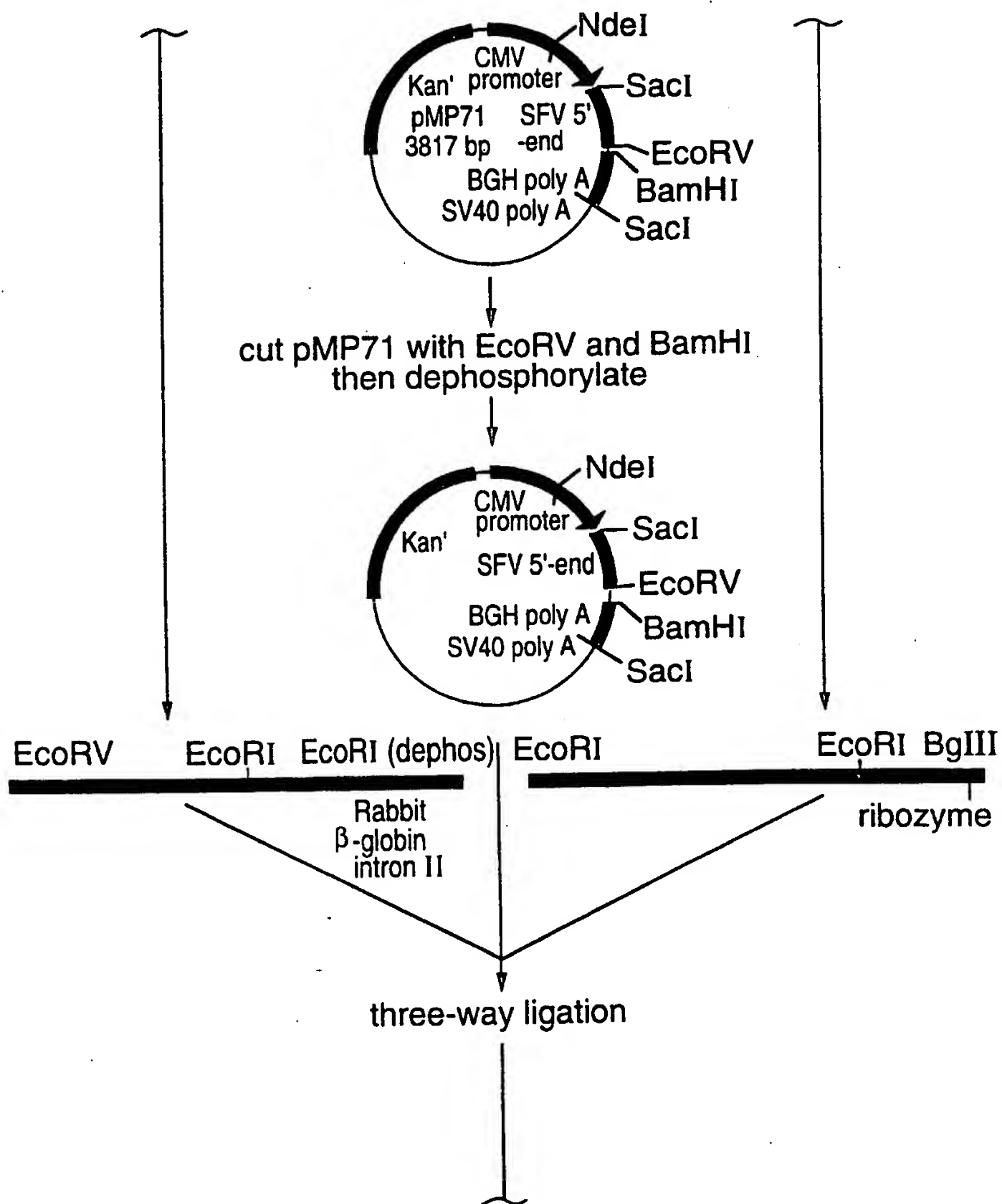


FIG.8B

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Construction of pMP76



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Construction of pMP76 (cont'd)

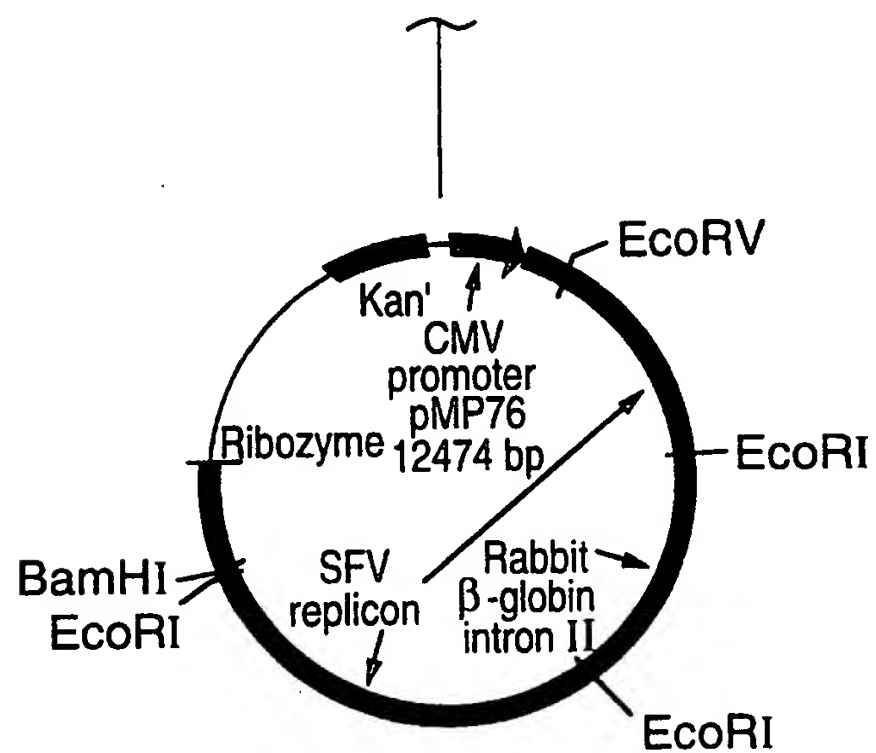


FIG.8D

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Construction of pMP53 & pMP54

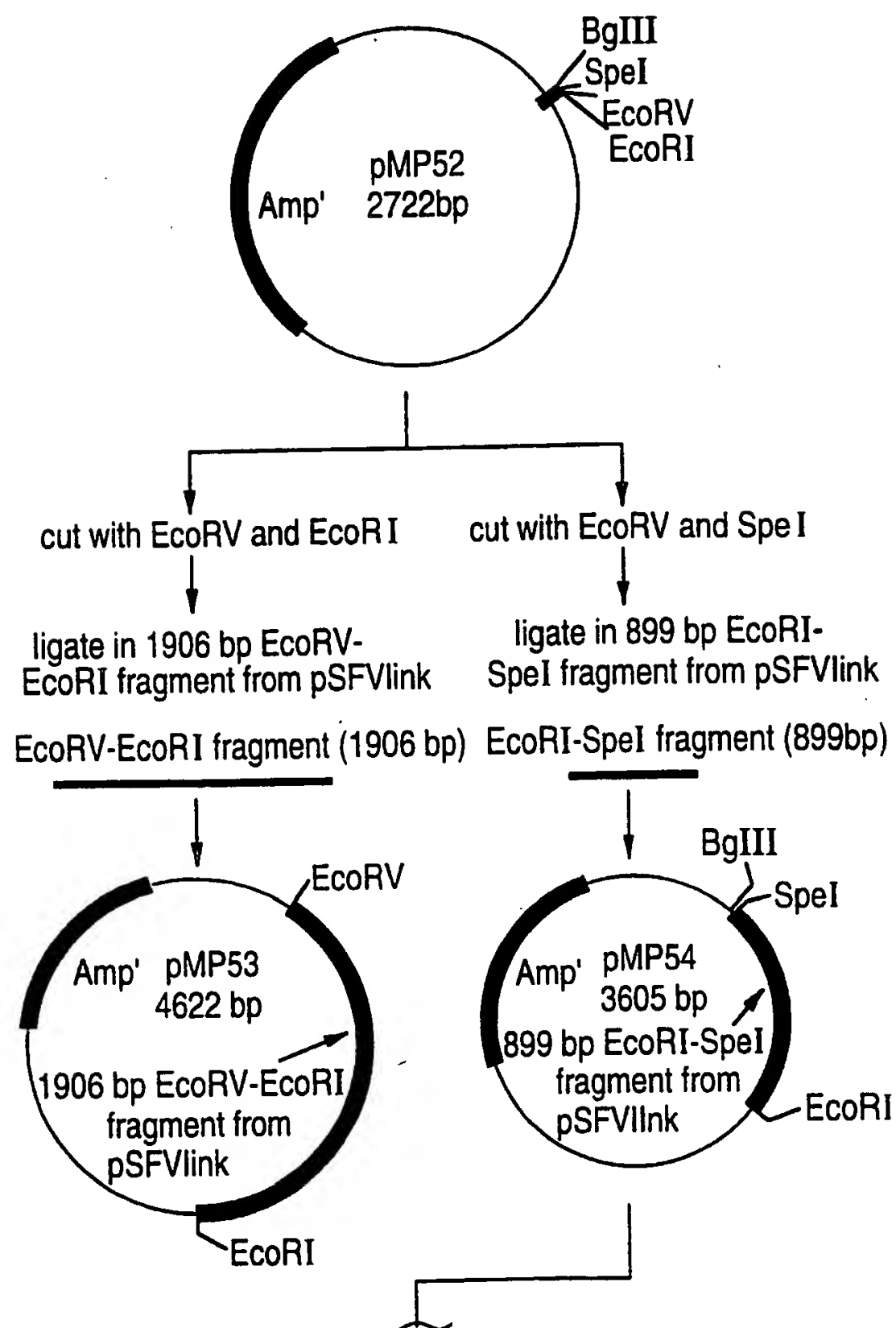


FIG.9A

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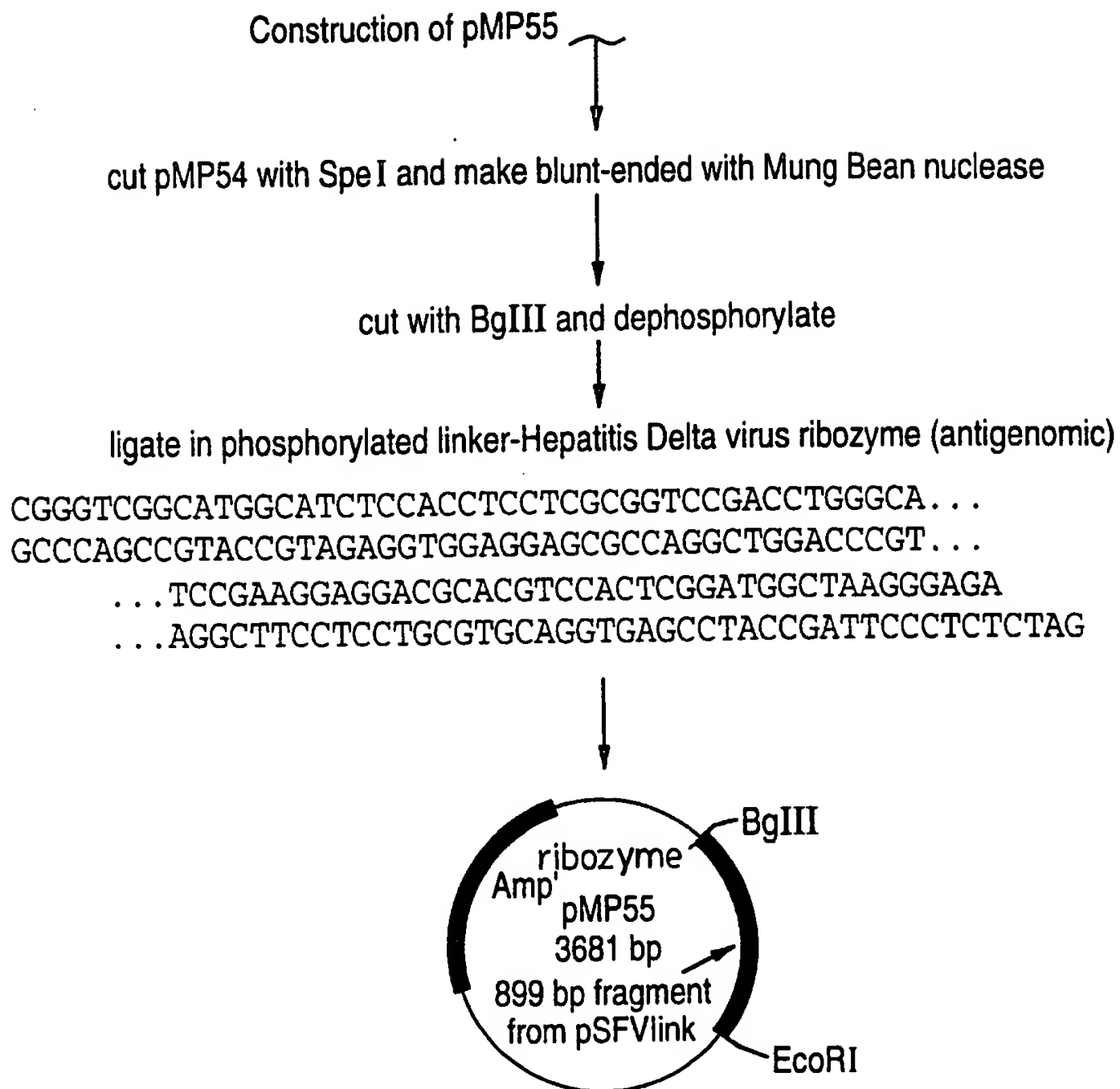


FIG.9B

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Construction of pMP52

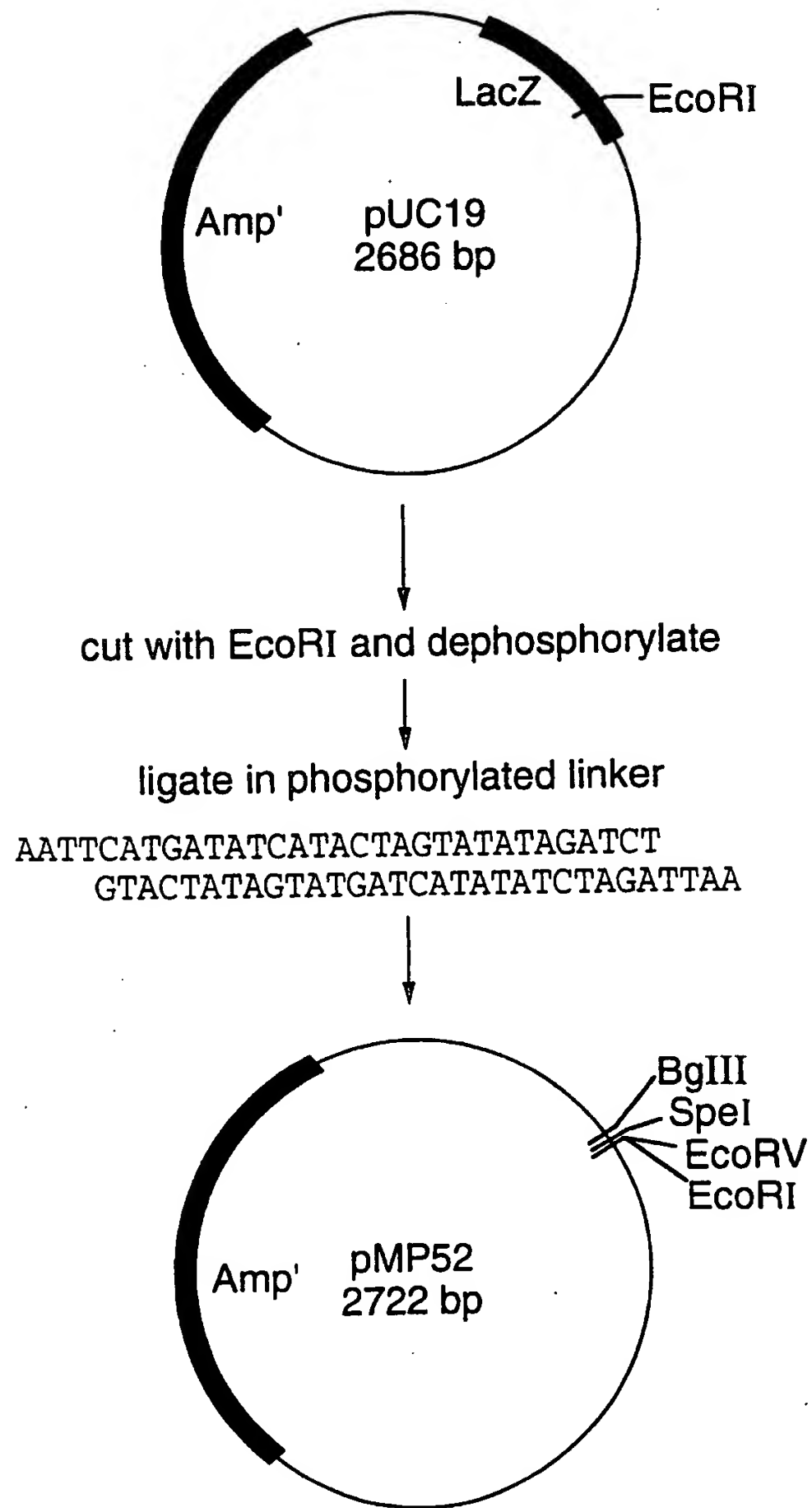


FIG.10

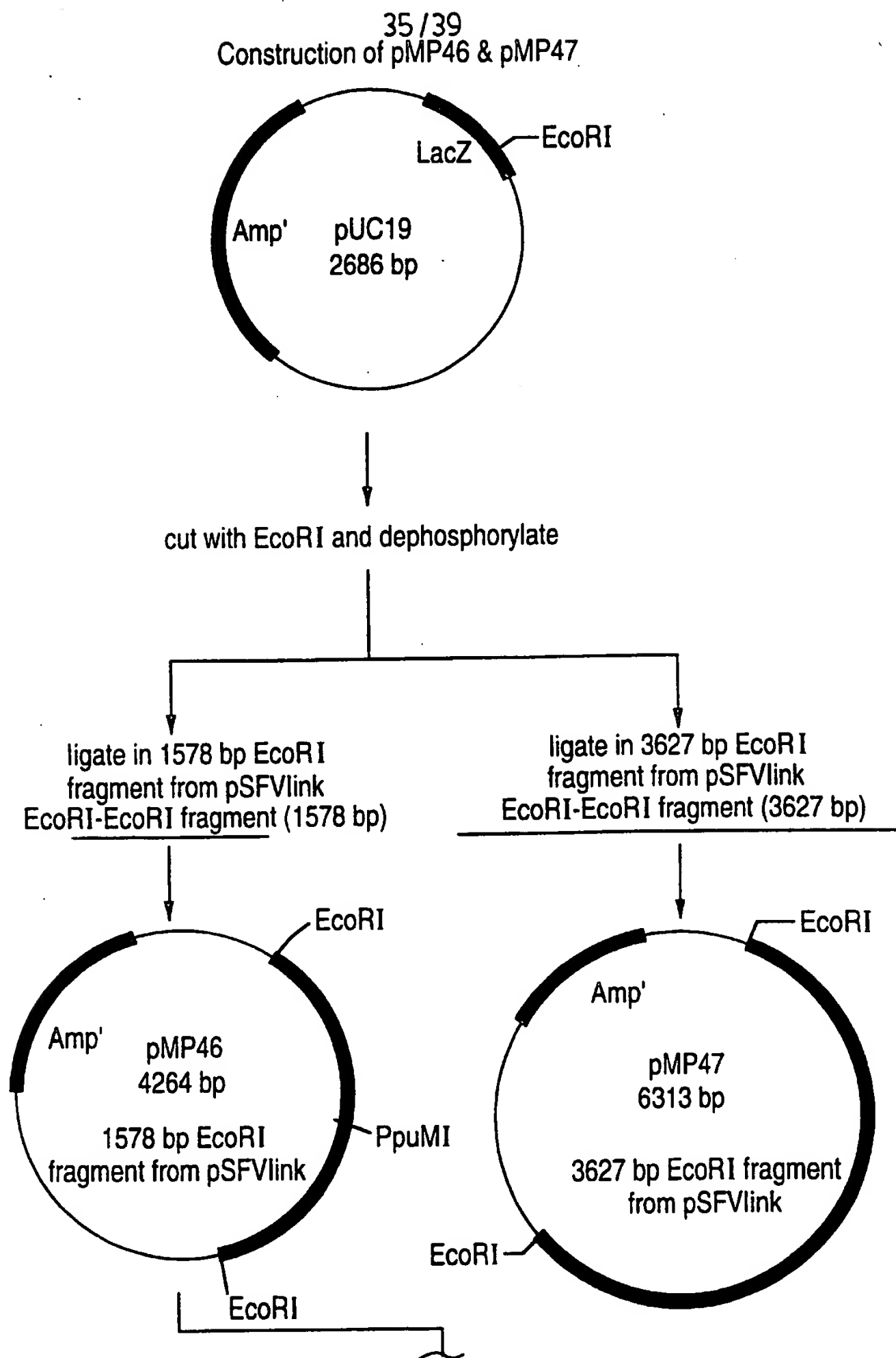


FIG.11A

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Construction of pMP70

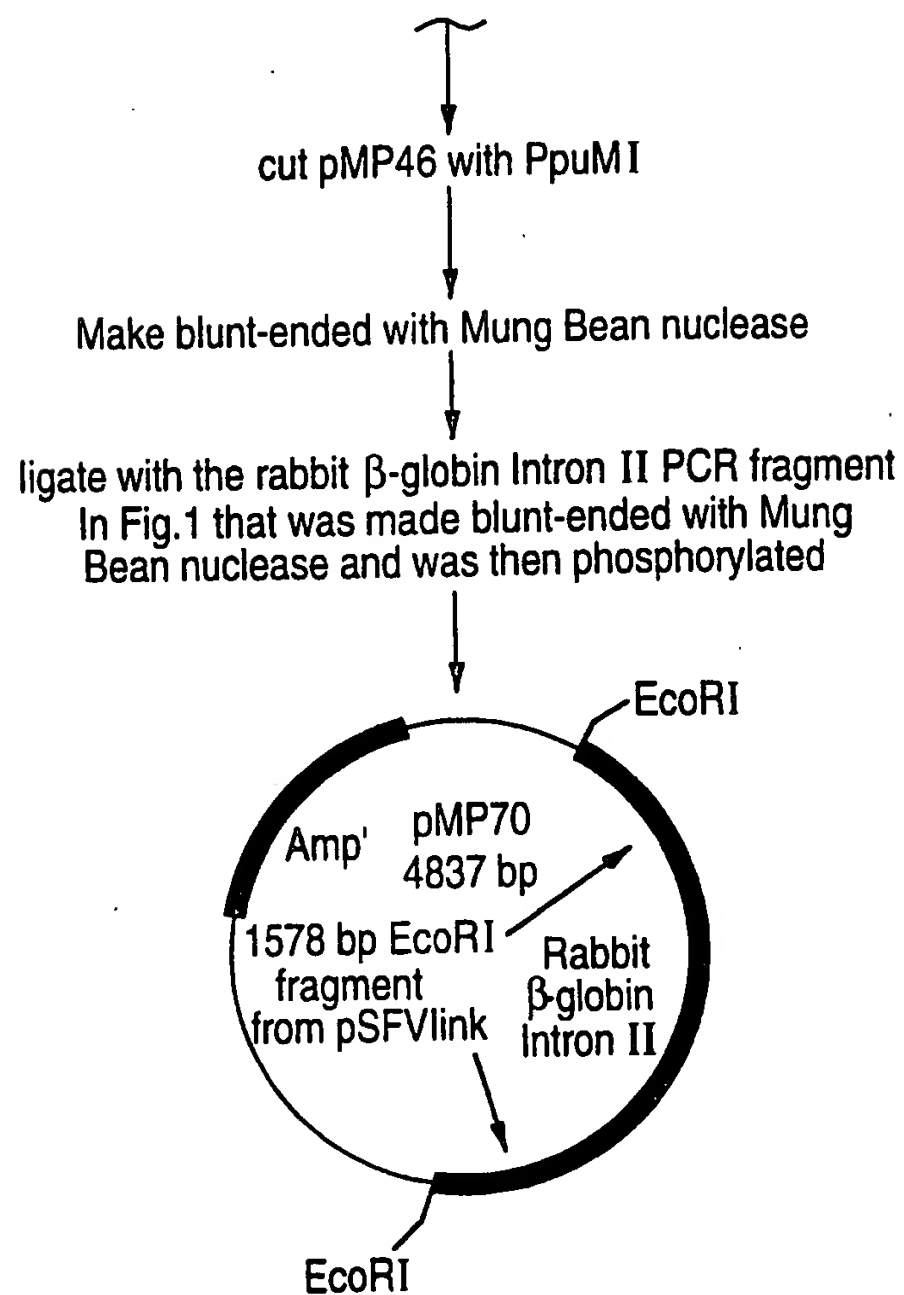


FIG.11B

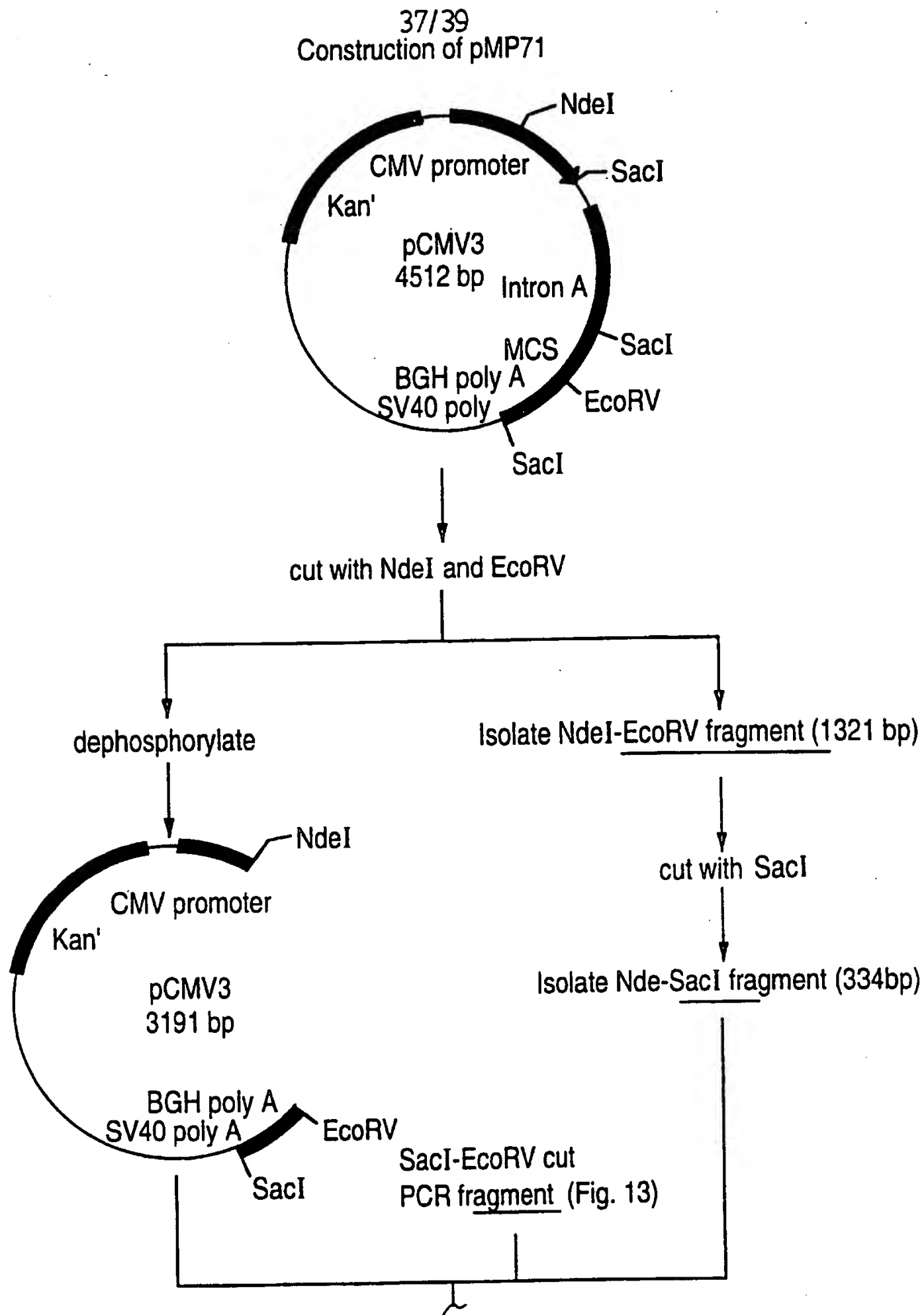


FIG.12A

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Construction of pMP71 (cont'd)

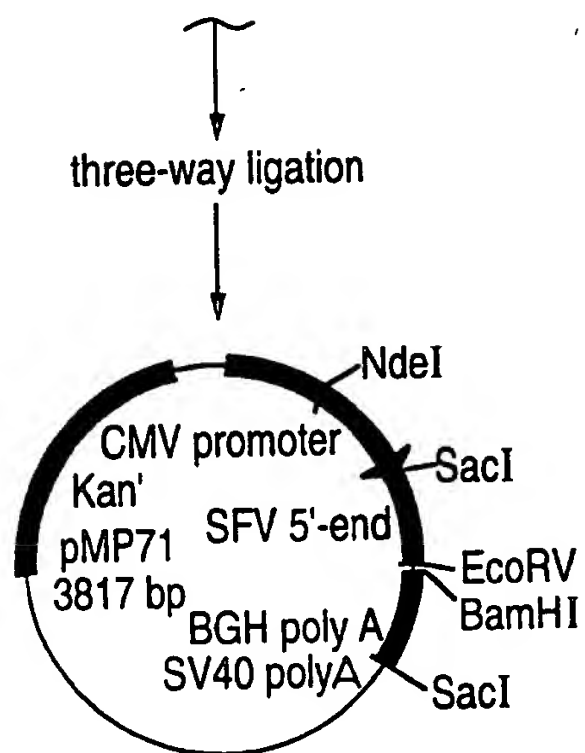


FIG.12B

FIG. 13

1 CGTTTAGTGA ACCGTATGGC GGATGTGTGA CATAACGAC GCCAAAAGAT 50
51 TTTGTTCCAG CTCCTGCCAC CTCGGCTACG CGAGAGATTA ACCACCCACG 100
101 ATGGCCGCCA AAGTGCATGT TGATATTGAG GCTGACAGCC CATTCATCAA 150
151 GTC'TTGCAG AAGGCATTTC CGTCGTTTCA GTGGAGTCA TTGCAGGTCA 200
201 CACCAAATGA CCATGCAAAT GCCAGAGCAT TTTCGCACCT GGCTACCAA 250^{39/39}
251 TTGATCGAGC AGGAGACTGA CAAAGACACA CTCATCTTGG AT 292³⁹

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/01065

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/86

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 95 27044 A (BIOPTION AB ; LILJESTROEM PETER (SE); GAROFF HENRIK (SE)) 12 October 1995 cited in the application see the whole document, especially page 8, lines 12-22 ---	1-14
Y	WO 96 40945 A (CONNAUGHT LAB ; LI XIAOMAO (CA); EWASYSHYN MARY E (CA); SAMBHARA SU) 19 December 1996 cited in the application see the whole document, especially page 6, lines 2-9; page 14, lines 15-21; and page 23, lines 18-23 ---	1-14
A	WO 96 17072 A (VIAGENE INC) 6 June 1996 see the whole document --- -/-	1-14

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Date of the actual completion of the international search

23 April 1999

Date of mailing of the international search report

03/05/1999

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NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
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Mandl, B

INTERNATIONAL SEARCH REPORT

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
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A	ZHOU X. ET AL.: "Self-replicating Semliki-Forest virus RNA as recombinant vaccine" VACCINE, vol. 12, no. 16, 1994, pages 1510-1514, XP002089524 cited in the application see the whole document ---	1-14
A	LILJESTROEM P. ET AL.: "A NEW GENERATION OF ANIMAL CELL EXPRESSION VECTORS BASED ON THE SEMLIKI FOREST VIRUS REPLICON" BIO/TECHNOLOGY, vol. 9, December 1991, pages 1356-1361, XP000616021 cited in the application see the whole document -----	1-14

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Information on patent family members

Intern. Application No
PCT/CA 98/01065

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